SOME EFFECTS OF SEASON, HABITAT, AND CLIPPING ON THE CHEMICAL COMPOSITION OF ANDROPOGON FURCATUS AND STIPA SPARTEA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 572

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Introduction

The chemical composition of forage plants as influenced by season, habitat, and grazing has been studied by many investigators. Such studies supply information on the comparative nutritive value of plants at various stages of development, under varying environmental conditions, and under grazing. In investigations concerned with the improvement and maintenance of the forage cover, a knowledge of the nutritive value of the plants is just as important as their palatability, yield, and reaction to grazing. The chemical composition of tops of forage plants frequently grazed may be closely associated with the relatively small amounts of roots and tops usually produced by these plants.

There has been little investigation of the chemical composition of many of the grasses of the true prairie, especially in eastern Nebraska. Big bluestem (*Andropogon furcatus* Muhl.) and needle grass (*Stipa spartea* Trin.) were selected for this work, as they are dominants in the prairie, are valuable pasture grasses, and are found as chief constituents of the best native hay in eastern Nebraska. The purpose of this study was to compare the seasonal trend of the chemical constituents of these two species in two habitats and to note the effects of clipping on the composition of native and seedling plants.

Review of literature

GORDON and SAMPSON (8) and WATKINS (20) have extensively reviewed the literature concerning the chemical composition of range plants. Many investigators have found that in range plants there is a decline in the percentage of crude protein and minerals and an increase in the percentage of crude fiber from the earliest appearance of leaf blades to maturity (4, 8, 13).

The storage of sugars and starch in certain grasses was found to be inversely related to the growth rate, but hemicellulose showed a rather continued percentage increase throughout the growth cycle of the plants (14, 15, 17).

STODDART (19) concluded that season had a far greater influence upon chemical composition of *Symphoricarpos rotundifolius* than did either site or soil.

Soil type affects the chemical composition of plants in part through its effect on availability of nutrients and water (4, 5, 7). The soil type may also influence the species composition of the flora and thereby indirectly the chemical composition of the forage.

BROWN (3) used thermo-regulated growth chambers in his investigations and showed that temperature has a marked effect on chemical composition of grasses.

Forage that regenerates after grazing or clipping has been found to be very suc-

culent, with high percentages of crude protein and low percentages of crude fiber (11, 18).

Materials

Big bluestem, a sod-former, is the most important native tall grass in eastern Nebraska. Here this grass, although deeply rooted, is typically an indicator of moist but well-aerated lowland, but it also occurs in limited amounts and small size on the dry uplands. It is of southern derivation and normally resumes growth at Lincoln about April 15. Its vegetative growth is normally complete by September, after which seed is usually produced in abundance.

Needle grass is found characteristically on dry uplands and, if elsewhere, on lighter types of soil. It produces bunches usually 2–4 inches in diameter, and the root system varies usually from 3 to 5 feet in depth. It is of northern extraction and usually renews growth in eastern Nebraska early in March and flowers and produces seeds in late May or early June. It does not grow vigorously thereafter until the cooler weather of early fall but continues growth until winter.

Two stations were selected in virgin prairie about 3 miles northwest of Lincoln, Nebraska. One station was on moderately low, gently sloping land, and the other area was about $\frac{1}{4}$ mile distant on a hill-crest. The soil at the lowland station was Wabash silt loam; that at the upland station, approximately 30 feet higher, was Carrington clay loam. Topsoil on the upland was about 2 feet in depth and on the lowland about 3 feet deep. In the lowland there was an abundance of big bluestem and a small area where needle grass was dominant; the upland had large amounts of needle grass, but only small bunches of big bluestem, scattered sparingly throughout.

Samples were taken throughout the growing season from the native grass at both stations, from areas on the lowland, which were repeatedly clipped at a height of 2 inches to simulate grazing, and from seedlings at both stations. The seedlings were grown in square-meter areas. In order to insure prompt, uniform germination and vigorous growth, the sod had been loosened to a depth of 2 inches, the coarser roots and rhizomes removed, and the soil well firmed, watered, and mulched after the seed had been planted.

Conditions for growth

The growing season of 1941 was characterized at Lincoln by a late spring, a wet April, moderate rainfall in May and June, and severe drought during July and August. Precipitation for April, May, and June was 3.12, 2.47, and 3.41 inches, respectively, or +0.59, −1.61, and −0.91 inches compared with the normal. The normal rainfall for July and August is 3.85 and 3.57 inches, but only 0.59 and 0.80 inches, respectively, fell during these months.

The mean monthly temperatures, April–August inclusive, are 54°.2, 68°.4, 72°.6, 78°.8, and 78°.5 F., respectively. Temperatures were all above normal during 1941, excess temperatures being +2°.7, +6°.7, +1°.2, +2°.3, and +4°.1 for the several months, respectively.

Evaporation from an open tank operated by the Weather Bureau at Lincoln was 7.1 inches in both May and June, 10.9 inches in July, and 10.2 inches in August.

Water content of soil, based on the total amount of moisture minus the hygroscopic coefficient at the various depths at each sampling, is shown in table 1. Several years of drought had preceded 1941; and, on March 31, soil on the upland was moist to a depth of only

29–39 inches and on the lower area to a depth of 49–57 inches.

On the upland, water was available for growth until late in June, after which very little occurred at any depth. Rainfall of 1 inch on June 27 was the last shower in excess of 0.2 inch until August 23, when 0.49 inch fell. Available water content at the lowland station was greater at all times and at all depths than on the upland. It, too, decreased sharply late in June, but water remained available, although often in small amounts, until August 31. These samplings for soil-moisture determinations were made near, but not in, the areas reserved for collecting plant materials. During the dry weather the area in the lowland from which the samples were taken for chemical analysis was supplied with enough water by sprinkling so that the grasses were able to continue growth. Those on the low ground completed their regular growth cycle and seeded abundantly. On the upland, water was applied only occasionally, and the big bluestem failed to produce flower stalks. Needle grass, however, produced seed in June. The showers, even in May and June, were poorly distributed for growth of seedlings. Therefore, the seedlings were watered as required to make possible adequate growth. An understanding of the development of the grass at each station at the times of sampling is essential to an interpretation of the chemical composition.

TABLE 1

WATER CONTENT OF SOIL IN EXCESS OF THE HYGROSCOPIC COEFFICIENT AT LINCOLN, NEBRASKA, DURING 1941

Depth in feet	Hygroscopic coefficient	March 31	May 10	May 24	June 20	July 11	July 24	Aug. 7	Aug. 31
	Upland Station								
0–0.5	8.4	18.8	15.3	14.0	4.1	−1.2	−2.7	−0.9	−1.8
0.5–1	9.6	12.3	11.3	8.3	5.1	−0.3	−1.3	−1.1	0.6
1–2	11.0	9.8	10.8	12.1	6.0	0.6	0.0	−1.1	−0.5
2–3	10.0	4.2	8.0	8.6	5.5	0.3	1.8	−0.9	0.6
3–4	7.5	4.2	.....	7.9	3.9	1.7	3.7	0.9	3.6
	Lowland Station								
0–0.5	9.4	28.2	24.5	21.1	10.6	1.0	3.2	1.7	0.8
0.5–1	10.6	18.0	17.8	16.5	11.8	1.2	0.2	1.2	0.7
1–2	12.0	10.2	15.0	13.2	9.9	2.1	1.4	0.5	0.2
2–3	12.4	8.7	13.5	12.4	10.0	4.9	6.3	1.0	0.3
3–4	12.3	7.5	.....	10.1	9.2	5.1	4.8	2.7	1.1
4–5	12.4	4.6	.....	6.2	.....	.....	0.0	3.6	2.5

Growth of the grass

Big bluestem

Development of big bluestem during the period of growth is shown by the data in table 2. The number of leaves was less on upland plants than on lowland after June 20 because of the extreme drought on the upland. There were green leaves on the watered lowland plants at

all times, but upland plants began to turn yellow or brown by July 10, and the leaf-tips dried despite watering. Actual decrease in general level of foliage of upland plants was also a drought effect; by August 7 the ends of the leaves had died back 2–5 inches and some whole shoots had died.[1] The plants were about one-third dried and had a dull-green color. Height of plants on lowland after July 10 was that of flower stalks; the general foliage level reached a maximum of about 26 inches. Plants on the upland produced no flower stalks, and by September 1 more than half of their leaves and stems were dead, and only a few of the green leaves were turgid. On August 7 and September 1 in the lowland, the flower stalks, each with five small leaves, were collected separately from the unelongated shoots in the same sods.

TABLE 2

GENERAL FOLIAGE HEIGHTS, NUMBER OF LEAVES, AND STEM DIAMETERS OF BIG BLUESTEM ON LOWLAND AND ON UPLAND AT TIMES OF COLLECTION OF SAMPLES

Date	Lowland			Upland		
	General foliage height (inches)	Average no. of leaves per culm	Range of stem diameter (mm.)	General foliage height (inches)	Average no. of leaves per culm	Range of stem diameter (mm.)
May 13	7	3	.....	6	3	.....
June 5	18	5	3–4	10	5	2–3
June 20	20	5	2–5	16	5	2–3
July 10	22	6	3–6	14	5	2–3
Aug. 7	32	6	3–6	14	5	2–3
Sept. 1	40	6	3–6	11	5	2–3

CLIPPED BLUESTEM ON THE LOWLAND

Certain patches of big bluestem on the lowland were clipped six times during the summer, but grass from the first clipping (May 13) was discarded, since it was the same as that of other lowland plants collected at this time. Material for the first analysis under this treatment was secured on June 5, when there were three or four leaf blades per shoot, projecting above the 2-inch-high clipping level, and the general height of the blades was 13 inches. By June 20 the plants had made an excellent recovery; most stems had two leaf blades above the clipping level, with a general height of 10 inches. At the clipping on July 10, the foliage height was about 12 inches, and there were usually four leaves more than 2 inches high on each culm. Since some terminal growing-points did not renew growth after the earlier clippings, the stand of grass was thinned but growing vigorously. At the fifth clipping on August 7, the sturdy stems of the still thinner stand usually had five leaves which had an average height of 10 inches. On September 1, much growth of basal tillers had occurred, and there were four broad leaves per stem and an average height of 7 inches. This grass rapidly regenerates after close cutting or grazing (21).

[1] All height measurements reported in this paper, unless otherwise specified, are the general heights of the foliage or flower stalks in their natural condition; leaves were never straightened out in order to obtain a measurement.

Bluestem seedlings

Seed was planted on April 26 at both stations. By June 20, when the plants were first clipped, the average foliage height on the lowland was 4.5 inches. There was an average of twenty-five seedlings per square decimeter, and the plants were in the sixth-leaf stage. Many seedlings had one or two tillers, but the latter were too short to be included in the samples, which were clipped at a height of 1.5 inches. On the upland the average plant height was an inch less than for lowland plants, and there were only fifteen plants per square decimeter. The number of leaves and degree of tillering, however, was about the same as for plants on the lowland. On July 24 there was a thick stand of much-tillered plants on the lowland. They had been watered weekly and were thriving. The seedlings had about six leaves and were once more 4.5 inches tall. Those on the upland were only 3.5 inches high, and some plants were much dwarfed, despite similar weekly watering. A third clipping was secured on September 1 from the seedlings on the lowland. The seedlings were then vigorously growing; their average height was 6 inches, and nearly all had either five or six leaves each.

Needle grass

Samples of *S. spartea* were collected seven times, April 23–September 1, inclusive. Foliage height was approximately the same at both stations at each sampling. On April 23, the general level of foliage was 5.5 inches, and the plants were in the third-leaf stage. By May 15 the general level of foliage was 13 inches, and the stems had an average of five leaves each. Inflorescences were present on some stems, but they were still inclosed in the leaf-sheaths. On June 5, flower stalks, which were harvested separately, were 36–48 inches tall when held erect. Each had three short leaves above the 2-inch cutting height; these were left on the stalks. The seeds were in the dough stage. The general level of foliage was 18 inches high, and each unelongated culm had three or four long, tapering leaves. No further increase in general level of foliage occurred. On June 20 the fruits had all been shed, and the flower stalks, which were dead and dry at least throughout their upper half, were not included in this or subsequent samples. On July 11, plants on the lowland were green and thriving; on the upland the leaves were rolled tightly because of drought. At the sampling on August 7, one-third of the leaves on lowland plants were dry or nearly dry, a usual midsummer phenomenon, and on the upland nearly one-half of the foliage was bleached white and dried. At the last sampling—September 1—the late-summer, half-dried condition prevailed on the lowland; on the upland many bunches were entirely dry, but others were one-third green.

Clipped needle grass

By May 13, twenty days after the first clipping, the plants had made much recovery. The general level of foliage was 11 inches, and there were a few flower stalks in an early stage of development. The general level of foliage was again 11 inches on June 5. Each stem usually had two narrow leaves projecting above the clipping level. Since the plants were suffering from too frequent removal of the foliage, the third sample was not taken until July 11. Each stem now had three leaves previously unclipped, with a general foliage level of 14 inches. Some bunches contained very few stems, while others were one-third filled. By August 7, little regeneration of the clipped grass

had occurred. Only ten to twenty-five stems scattered throughout the large bunches renewed growth. The general height of foliage was about 7 inches, and each culm had an average of three to five leaves each. On September 1 the general level of foliage was 6 inches, and there were three leaves or parts of leaves per stem.

Seedling needle grass

The needle grass, planted April 26, grew more rapidly in the warmer and drier upland soil, where it had attained a general level of foliage of 7 inches when clipped on June 20. Seedlings were then in the third- or fourth-leaf stage. Most plants had one or two tillers, 1.5–2.5 inches in height. The stand was open, only about 8 plants occurring per square decimeter. When the needle grass on the lowland was clipped on July 3, it had attained approximately the same size and density of stand as that described on the upland. Needle grass on the upland regenerated to such a small degree that it was not clipped again. The grass on the lowland was harvested on September 1, when the plants had a foliage level of 5 inches and an average of three leaves per culm.

Methods

Collection of samples

Each composite sample for chemical analysis consisted of plants taken in at least five different places in the local experimental area. Further samples were never taken from the same place, thus assuring the natural growth and maturing of the tissues included in each sample.

In the clipping experiments, parts of many plants were included, as an area of about 1 square meter was clipped on each collecting date. The seedlings likewise were all clipped at each of the several times of collecting, whether or not all material was used for the chemical analysis.

Samples were taken between 10:00 and 11:00 A.M. and in a definite sequence. They included the entire aerial portion of the plant, unless otherwise specified. The grass was placed in paper bags and taken at once to the laboratory, where it was then killed by being subjected to a temperature of approximately 100° C. It was placed on a 2-mm. mesh screen, and heat was applied from below by an electric heater with a large reflector. This arrangement permitted constant movement of large amounts of hot dry air. After 5–8 minutes the temperature was decreased to 70° C. for 15 minutes, and the final drying was in an electric oven at that temperature.

Chemical analysis

When completely dry, the grass was finely ground and placed in tightly stoppered bottles. Duplicate samples were used for the determinations, and the analytical methods used were those described by Loomis and Shull (12). The reduced-iron method adapted by Pucher was used for determination of total soluble nitrogen.

Results

Big bluestem

It is emphasized that all analytical data in this investigation are expressed as percentage values and not as absolute amounts.

On each sampling date throughout the season, established plants on the lowland had the highest percentage of total sugars, clipped plants on the lowland the lowest, and upland plants had an intermediate percentage (table 3). Seedlings

on the upland contained about the same percentage of sugar as established upland plants, but the percentage sugar content of lowland seedlings was small.

Established plants on the upland contained the highest percentage of starch of seedling plants in the two habitats was about equal.

The percentage sugar-starch content of established lowland grass was less than that on the upland early in the season, but it was greater on the lowland after the latter part of June, when drought became severe on the upland (fig. 1). Upland seedlings had a higher percentage content of this fraction than did those growing on the lowland. The lowland clipped plants had a gradually decreasing sugar-starch fraction during most of the season.

TABLE 3

PARTIAL CHEMICAL ANALYSIS OF ABOVE-GROUND PARTS OF BIG BLUESTEM AT SIX STAGES OF DEVELOPMENT, EXPRESSED AS PERCENTAGES OF OVEN-DRY WEIGHT

Plants	Date collected	Total sugars	Starch	Total sugars and starch	Hemicellulose	Soluble nitrogen	"True" protein	Total ash
Upland	May 13	1.7	6.8	8.5	13.8	0.30	12.5	8.5
	June 5	2.9	6.4	9.3	18.5	.17	7.7	5.3
	June 20	1.4	6.5	7.9	17.6	.16	6.1	5.5
	July 10	3.4	3.9	7.3	21.6	.12	4.8	5.2
	Aug. 7	1.8	3.7	5.5	21.4	.10	3.2	4.5
	Sept. 1	1.0	3.8	4.8	20.6	.09	2.6	5.1
Lowland	May 13	1.9	4.6	6.5	13.2	.41	15.0	9.5
	June 5	3.2	4.1	7.3	19.5	.14	7.8	6.4
	June 20	1.9	5.8	7.7	20.3	.13	5.6	6.2
	July 10	4.5	4.1	8.6	21.6	.11	4.2	5.9
	Aug. 7	3.1	3.4	6.5	22.8	.09	3.2	5.4
	Aug. 7*	4.0	3.9	7.9	23.5	.09	2.4	4.3
	Aug. 7†	1.9	2.6	4.5	21.8	.10	4.5	7.1
	Sept. 1	2.0	4.7	6.7	23.8	.08	2.5	5.7
	Sept. 1*	2.1	4.8	6.9	24.2	.07	2.2	4.7
	Sept. 1†	1.8	4.5	6.3	23.0	.08	3.1	7.2
Lowland, clipped	May 13	1.9	4.6	6.5	13.2	.41	15.0	9.5
	June 5	2.3	3.8	6.1	15.9	.16	9.5	8.0
	June 20	0.8	3.9	4.7	13.7	.27	11.6	9.7
	July 10	1.6	2.0	3.6	18.9	.16	8.3	7.7
	Aug. 7	1.6	1.8	3.4	18.7	.14	7.3	7.2
	Sept. 1	0.9	4.0	4.9	18.5	.14	7.1	7.6
Upland seedlings	June 20	1.6	4.9	6.5	12.0	.16	10.1	7.1
	July 24	3.0	3.8	6.8	15.5	.18	7.2	6.0
Lowland seedlings	June 20	0.9	4.5	5.4	12.7	.19	8.3	8.4
	July 24	2.0	3.7	5.7	18.6	.16	7.2	7.7
	Sept. 1	1.5	3.2	4.7	15.5	0.13	7.1	8.2

* Analysis of stems and flowers.

† Analysis of leaves.

The percentage of hemicellulose increased generally during the growth cycle of the plants (fig. 1). Established plants on the lowland usually contained a higher percentage of hemicellulose than the corresponding upland plants. The percentage content of this fraction in seedlings at the time of sampling was less than in established plants growing near by. The clippings from established grass at the lowland station contained a smaller percentage of hemicellulose than did the unclipped plants.

On May 13, established lowland plants contained more total soluble nitrogen than did corresponding upland plants; but, after June 5, upland plants had a higher percentage of their weight in this fraction, although after June 20 the difference was probably not significant (fig. 1). Seedling plants had, in general, a higher percentage of soluble nitrogen than did the established plants. Clipped plants on the lowland, with one exception, had a higher percentage of soluble nitrogen than did unclipped plants.

The graph showing percentage changes of "true" protein is, in general, similar to the graph for total soluble nitrogen (fig. 1).

Established plants had a relatively high percentage of ash at the first sampling (fig. 1). Lowland plants had consistently about 0.5–1.0 per cent higher total ash content than did upland plants. Seedlings had a higher percentage ash content than the corresponding established plants did, and established plants on the lowland, which were clipped throughout the season, maintained a high percentage level of total ash.

The chemical composition of flower stalks and leaves at the last two sampling dates is shown in table 3. On August 7 each inflorescence was about one-half exserted, and by September 1 they were partly to fully expanded. On August 7 the flower stalks contained almost twice the percentage of sugar and starch as did the leaves, but by September 1 the percentages were approximately equal. There was slightly more hemicellulose on a percentage basis in stalks than in the leaves. On August 7 the ratio of percentage of "true" protein in flower stalks to percentage in the leaves was about 1:1.9, but by September 1 this ratio had been reduced to 1:1.4. Leaves contained about 1.6 times as much total ash as did the flower stalks on a percentage basis.

NEEDLE GRASS

The percentage content of total sugars was relatively high early in the season (table 4), and established plants on the upland usually had the highest percentage content of total sugars. These data indicate that the percentages of starch and of sugar plus starch decreased as the season advanced and that no one treatment had the highest percentages of these compounds at all samplings (fig. 2).

Established plants on the upland contained a higher percentage of hemicellulose than did the corresponding lowland plants (fig. 2). Clippings from established and seedling grass usually contained smaller percentages of hemicellulose than did unclipped plants.

Graphs showing percentage changes of total soluble nitrogen and "true" protein in the grass are, in general, similar (fig. 2). The percentage content of these constituents decreased with the advancing season, and all clipped plants had a higher percentage of their weight in these fractions than did unclipped plants.

Established plants on the lowland maintained a higher percentage ash content than did either established plants on the upland or clipped established plants on the lowland (fig. 2). Clipped seedlings

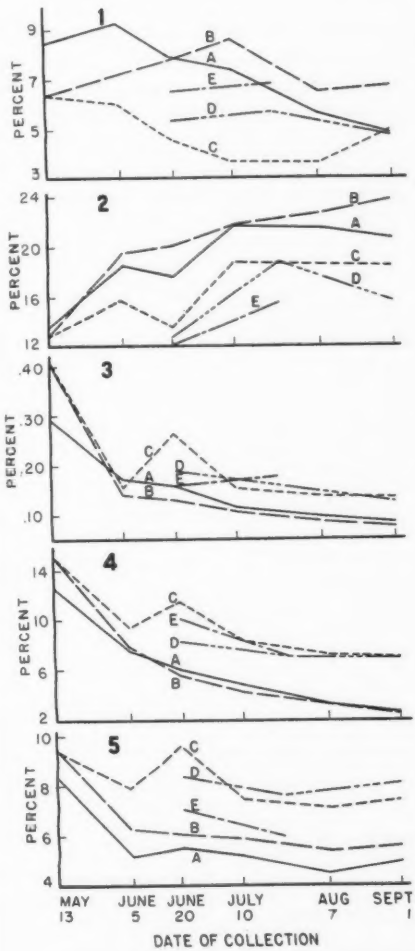

FIG. 1.—Total percentages (dry-weight basis) of (1) sugars and starch, (2) hemicelluloses, (3) soluble nitrogen, (4) "true" protein, and (5) ash in big bluestem at several stages of growth, clipped and not clipped. *A*, established plants on the upland; *B*, same on the lowland; *C*, same clipped plants on the lowland; *D*, clipped lowland seedlings; and *E*, clipped upland seedlings.

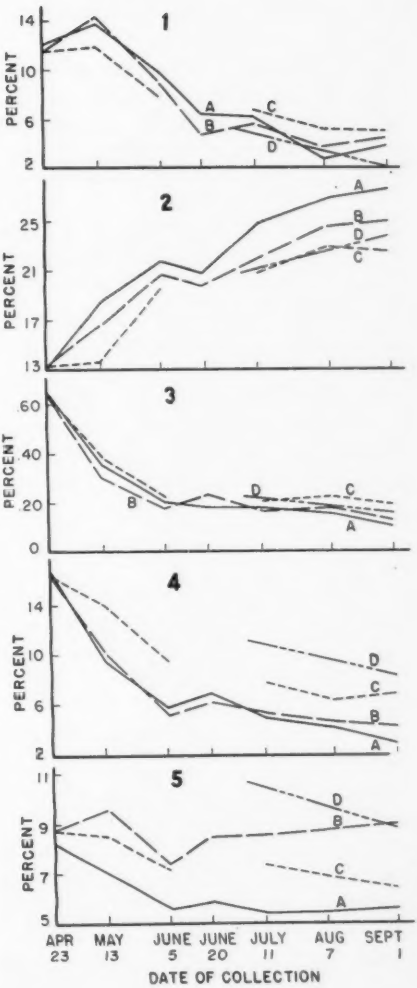

FIG. 2.—Total percentages (dry-weight basis) of (1) sugars and starch, (2) hemicelluloses, (3) soluble nitrogen, (4) "true" protein, and (5) ash in needle grass at several stages of growth, clipped and not clipped. *A*, established plants on the upland; *B*, same on the lowland; *C*, same clipped plants on the lowland; and *D*, clipped lowland seedlings.

usually had the highest percentage ash content.

The chemical composition of flower stalks and leaves of plants on the upland and on the lowland at the sampling on June 5 is shown in table 4. Flower stalks had a higher percentage content of sugar-starch but lower percentages of hemicellulose, soluble nitrogen, "true" protein, and total ash than did the leaves.

TABLE 4

PARTIAL CHEMICAL ANALYSIS OF ABOVE-GROUND PARTS OF NEEDLE GRASS AT SEVEN STAGES OF DEVELOPMENT, EXPRESSED AS PERCENTAGES OF OVEN-DRY WEIGHT

Plants	Date collected	Total sugars	Starch	Total sugars and starch	Hemicellulose	Soluble nitrogen	"True" protein	Total ash
Upland	April 23	6.3	5.7	12.0	13.1	0.65	16.8	8.3
	May 13	7.5	6.1	13.6	18.3	.35	9.6	7.0
	June 5	4.9	4.9	9.8	21.5	.20	5.7	5.6
	June 5*	5.4	5.6	11.0	21.2	.18	4.4	5.4
	June 5†	3.6	2.8	6.4	21.9	.24	8.8	6.0
	June 20	2.4	4.0	6.4	20.8	.19	6.9	5.9
	July 11	3.7	2.5	6.2	24.6	.18	5.1	5.4
	Aug. 7	0.8	1.8	2.6	26.7	.15	4.4	5.5
	Sept. 1	0.7	3.1	3.8	27.5	.10	2.9	5.6
Lowland	April 23	6.1	5.5	11.6	13.2	.64	16.6	8.8
	May 13	7.5	6.7	14.2	16.5	.30	10.1	9.6
	June 5	4.3	4.7	9.0	20.6	.18	5.3	7.3
	June 5*	4.5	5.0	9.5	20.5	.16	4.5	6.8
	June 5†	3.5	3.8	7.3	20.8	.22	7.7	8.8
	June 20	1.3	3.3	4.6	19.6	.23	6.2	8.5
	July 11	2.1	3.1	5.2	21.8	.17	5.5	8.5
	Aug. 7	0.5	3.5	4.0	24.5	.16	4.8	8.8
	Sept. 1	0.6	3.8	4.4	24.9	.12	4.4	9.0
Lowland, clipped	April 23	6.1	5.5	11.6	13.2	.64	16.6	8.8
	May 13	6.5	5.3	11.8	13.7	.37	13.9	8.5
	June 5	3.3	4.4	7.7	19.6	.21	9.6	7.2
	July 11	2.5	4.0	6.5	20.9	.20	7.9	7.3
	Aug. 7	1.7	3.3	5.0	22.8	.22	6.4	6.9
	Sept. 1	0.7	4.0	4.7	22.6	.18	7.0	6.4
Upland seedlings	June 20	0.9	7.8	8.7	16.2	.25	13.0	9.3
Lowland seedlings	July 3	1.3	3.9	5.2	20.9	.22	11.3	10.7
	Sept. 1	0.6	1.4	2.0	23.8	0.15	8.5	8.9

* Analysis of stems and flowers. † Analysis of leaves.

Discussion

The rapid percentage decrease of crude protein in established, unclipped plants shortly after the early leaf stage, the further slight decrease as the season advanced, and the gradual increase of the hemicellulose fraction throughout the growth cycle are in agreement with the results of other investigators (4, 8).

Early in the season unclipped established big bluestem on the upland usually had a higher percentage content of sugar plus starch than did big bluestem on the lowland. After June 20, however, when drought became severe on the upland, the situation was reversed. When soil moisture became depleted on the upland,

the tips of the leaves of big bluestem began to die back and dry up, resulting in decreased photosynthetic activity. The degree of development of established unclipped needle grass on the upland and the lowland was quite similar throughout the season of growth. On June 11, however, leaves of needle grass on the upland were rolled tightly because of drought, and, later, more drying of foliage occurred on the upland than on the lowland. This may partially account for the lower sugar-starch percentage in needle grass on the upland at the last two times of sampling.

It has been shown that part of the change in composition of forage with advancing maturity is the result of leaching of soluble constituents (9). The sugar fraction could be readily leached from the dried grass leaves. Although precipitation was abnormally low during the latter part of the growing season, the rains on June 27 and August 23 of 1.00 and 0.49 inches, respectively, may have caused considerable leaching. Since much drying-out of leaves occurred in the unclipped established plants on the upland and less drying occurred on the lowland, more leaching would be expected from the former plants.

It was noted that early in the season when the percentage of starch in big bluestem was high, the percentage of sugar was low, but that, as the season progressed, the situation was reversed. Perhaps a partial explanation for this is that these fractions are closely interrelated. Investigations with frequent samplings, however, are necessary for proof that these are seasonal changes and not those of daily or short duration.

In reviewing the literature, EATON (6) noted that opinions are still in conflict as to whether acid-hydrolyzable carbohydrates (hemicellulose) are used in metabolism or whether they are permanent structural constituents of the cell wall. MCCARTY (14, 15), in experiments with the wild oat (*Avena fatua*) and mountain brome (*Bromus carinatus*), found indications that the acid-hydrolyzable hemicellulose is largely employed as a structural material even by clipped plants. The percentage of hemicellulose in the unclipped big bluestem and needle grass generally increased during the advancing season, which suggests that this fraction is not used, at least in large amounts, as a reserve food. The percentage of hemicellulose in established upland big bluestem decreased slightly during the interval from June 5 to June 20. Big bluestem on the upland grew rapidly during this period, growing 6 inches in average foliage height. This rapid growth may have resulted in the addition of much succulent tissue, with a lower percentage of hemicellulose. The usually greater hemicellulose percentage in big bluestem on the lowland than on the upland may be due to the more stemmy nature of the former plants. Stems of big bluestem contain a slightly larger proportion of hemicellulose than do the leaves.

In established unclipped plants the proportion of available carbohydrates to available nitrogenous compounds progressively increased with the stage of development of the plant. These data are in agreement with the findings of KRAUS and KRAYBILL (10), who showed that when plants were vigorously vegetative but unfruitful, the proportion of available carbohydrates to nitrogen was relatively low, but in plants which were vigorous and also reproductive the relative proportion of these was intermediate.

The clippings from seedlings clipped several times during the growing season were in a succulent condition. This was reflected in the high percentages of sol-

uble nitrogen, "true" protein, and low percentage of hemicellulose. Foliage which grows after clipping or grazing is approximately as succulent as is that in the spring.

Clippings from the established plants which were periodically clipped on the lowland were also succulent and showed high percentages of soluble nitrogen and "true" protein and a low percentage of hemicellulose at the times of sampling. The rapid decrease in the percentage of the sugar-starch fraction in the clipped grasses during their development probably resulted from the rapid conversion of the products of photosynthesis into structural materials. McCARTY (14) reported that in the wild oat there was low food (sugar and starch) concentration in the herbage regenerated after cutting. He showed in mountain brome in California that the annual herbage growth and yield are products of carbohydrates manufactured currently in the herbage (15). The carbohydrates in the roots and stem bases of mountain brome were at a minimum shortly after snow disappeared in spring.

The results from clipped big bluestem and needle grass indicate that the chemical composition varies during the season, even though the herbage is maintained in a succulent condition by repeated clipping. ARCHIBOLD and NELSON (1) have stated: "Grass kept in the vegetative stage by grazing may be quite different in chemical composition in midsummer from what it was in the spring. The seasonal factors, rainfall, temperature, sunshine, and length of day, exert their influence irrespective of the stage of growth of the plant."

Since the total soluble nitrogen, "true" protein, and total ash percentages were higher in grasses remaining in the leaf stage than in plants that had developed flower stalks, it is probable that the former plants are more nutritious forage for animals. CLARKE and TISDALE (4) found that, in junegrass (*Koeleria cristata*), common speargrass (*S. comata*), and grama grass (*Bouteloua gracilis*), leafage was generally richer in crude protein, total ash, calcium, and phosphorus, and lower in percentage of crude fiber than the culms.

An explanation of the causes for the difference in chemical composition of established plants on the upland and the lowland is difficult, because possible differences in several factors of the environment—water content of soil, humidity, light, temperature, soil solutes, and soil air—may have directly affected the plants. The chemical composition of plants is closely associated with their stage of growth. In this experiment the stages of growth of big bluestem varied greatly between plants on the upland and on the lowland; only on the lowland did flowering occur. The differences in degree of development of needle grass in the two habitats was slight, but more drying of foliage occurred in plants on the upland. Since the supply of available water was probably the most critically controlling environmental factor in determining the degree of development and drying of the plants, varying water content between upland and lowland was perhaps the primary factor associated with the chemical differences between the plants.

The variation in height and density of stand of big bluestem on the upland, as contrasted with its growth on the lowland, may have been of importance in the chemical differences. In the taller and denser stand on the lowland the lower leaves would be shaded, with corresponding limitations on their rate of photosynthesis. This might bring about variation

in the entire chemical composition of the plant.

The scattered plants of big bluestem on the upland might be genetically different from plants on the lowland. Big bluestem grows normally in low moist soil, and the plants which persisted on the dry hill-crests might be a drought-resistant strain. The scattered bunches of needle grass on the lowland might, on the other hand, be a strain adapted to a moist environment. If this were true, the same species in the two habitats might have differing chemical composition even if grown under identical conditions. The use of identical clones in each area would eliminate this possibility for error.

It is generally agreed that frequent clipping of tops of plants usually reduces the yield both of tops and of roots. In eastern Nebraska, BISWELL and WEAVER (2) found that the dry weight of tops from sods of big bluestem clipped seven times during the season of growth was only 14.5 per cent of that of unclipped plants and that the dry weight of roots from clipped plants was only 5.3 per cent of that of controls. The low percentage content of the sugar-starch fraction usually present in the clipped big bluestem and needle grass was probably associated with a decreased production of tops and roots.

These data may be of significance in estimating the value of forage plants growing in different habitats. For example, if the chemical composition of big bluestem or needle grass growing on the upland and lowland is known, one should be able to estimate their relative forage values when the analytical data are used in conjunction with the yields and palatability of the grasses in the two habitats. Digestion trials should be used in conjunction with analytical data, as it has been shown that chemical analysis when used alone may sometimes be misleading. Forages of similar composition may have different digestibilities and thus be of unequal value to the animal (16).

A knowledge of chemical composition of clippings from cut forage is important in comparing seasonal value of the clippings with that of plants allowed to grow to maturity. A study of chemical composition would be a valuable adjunct to all experiments in which the reaction of forage to clipping or grazing is studied.

Summary

1. The seasonal trend of certain chemical constituents of established big bluestem (*A. furcatus*) and needle grass (*S. spartea*) growing on lowland and upland habitats in eastern Nebraska was studied. The effects of periodic clipping on the composition of established and seedling plants were also investigated.

2. The growing season was characterized by a late spring; sufficient rainfall in April, May, and June; and severe drought in July and August. Mean monthly temperatures were all above normal. After late June little soil moisture available for growth occurred on the upland. On the lowland, water remained available, at least in small amounts, throughout the investigation.

3. Established big bluestem plants on the lowland became taller than those on the upland. The latter showed severe drought effects early in July, and flowering occurred only in the lowland plants. The degree of development of needle grass in the two habitats was similar, although more drying of foliage occurred in plants on the upland. During the flowering stage, grasses with flower stalks and grasses remaining in the leaf stage were separately collected.

Established grasses on the lowland regenerated after five cuttings. Seeds plant-

ed on the upland and lowland produced seedlings, which were clipped from one to three times.

4. The sugar plus starch percentage was greater in big bluestem on the upland than on the lowland early in the season, but later the situation was reversed. The percentage decreased in big bluestem on the upland when the leaves began to dry up. This drying may have reduced photosynthesis and may have permitted the soluble sugars to be leached from the leaves.

The hemicellulose percentage in big bluestem was usually higher in established plants on the lowland, perhaps because of their more stemmy nature. Needle grass on the upland, however, had a higher percentage of hemicellulose than lowland plants. Indications were that hemicellulose is not used as a reserve food.

5. Varying available water content between the upland and the lowland was probably the primary factor in causing the established unclipped plants in the two habitats to be chemically different, since the supply of available water was probably the most critically controlling factor in determining the degree of development and drying of the plants.

6. Clippings from established plants which were periodically clipped were succulent, and their percentages of soluble nitrogen and "true" protein were high, but their percentage content of hemicellulose was low. Clippings from seedlings showed generally similar results.

7. The soluble nitrogen, "true" protein, and total ash percentages were higher in grass remaining in the leaf stage than in plants with flower stalks, but the sugar-starch percentage was greater in the culms.

8. This investigation may be of value in comparing the forage value of big bluestem and needle grass in different habitats and under grazing.

The writer expresses his appreciation to members of the Department of Botany of the University of Chicago for helpful advice throughout the course of the research.

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MODE, SITE, AND TIME OF INITIATION OF HYPOCOTYLEDONARY BUD PRIMORDIA IN LINUM USITATISSIMUM L.[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORIES 573

GEORGE K. K. LINK AND VIRGINIA EGGERS

Introduction

In the course of a study by EGGERS (6, 7) of the role of carbohydrate and nitrate-nitrogen nutrition in hypocotyledonary bud formation and development in the Bison variety of *Linum usitatissimum* L., tests were begun in 1936 to investigate the effects of auxins upon the development of hypocotyledonary bud primordia into buds and shoots. The results of those studies will be communicated in a later paper. During the early phases of those studies we found it necessary to reinvestigate the problems of mode, site, and time of initiation of these bud primordia in both nondecapitated and decapitated hypocotyls of the flax plant. The results of the latter studies are reported in this paper.

The literature bearing on this topic is not very extensive. BURNS and HEDDEN (4) in 1906 reported results of their studies of the conditions which affect the appearance of hypocotyledonary buds in several plants, including *L. usitatissimum*. TAMMES (10) corroborated their findings as to the occurrence of such buds in decapitated flax hypocotyls, and ADAMS (1), apparently unaware of these reports, announced the discovery of such buds for decapitated hypocotyls of flax. BEALS (3) included the formation of hypocotyledonary buds in flax in her study of the histogenesis of regenerated organs. NEUBERT (8) made extensive studies of the regenerative activities of several species of flax, including *L. usitatissimum*. CROOKS (5) in the course of a histological study of the seedling of flax also investigated its regenerative activities and made a detailed study of the histogenesis

[1] This is the second of a series of papers reporting the results of studies of hypocotyledonary and epicotyledonary vegetative buds and wound tissue of *Linum usitatissimum* L. by George K. K. Link and Virginia Eggers, which have extended from 1934 to date. The work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

of its hypocotyledonary buds. A monograph dealing with the development of subcotyledonary shoots and their significance in the growth forms of plants was published by RAUH (9). He supplemented CROOK's findings with results derived from investigation of the histogenesis of hypocotyledonary buds in *L. usitatissimum* L. and *L. tenuifolium* L.

Materials and methods

After a series of preliminary studies, seed of the Bison variety of *L. usitatissimum* was planted in May, 1939, in soil and in sand in pots and between moist filter paper in Petri dishes. The seedlings emerged from the soil and sand on the 4th day. On the 5th day some seedlings were decapitated immediately below the cotyledons, the others were left uncut as controls. Samples of the uncut hypocotyls of the soil and sand plantings were collected every day for 15 days, and of the filter paper set for 3 days. In another set, samples were taken on the 36th day. The first samples of the decapitated hypocotyls were collected 2 days after decapitation (7-day-old plants) and every second day thereafter through the 10th day after decapitation. Samples also were taken 10 days after decapitation of 36-day-old plants.

The specimens taken were fixed in Crook's modification of Navashin's solution. As modified, this consists of solution A (7 cc. glacial acetic acid, 1 gm. chromic acid crystals, and 92 cc. distilled water) and solution B (30 cc. formalin and 70 cc. water), mixed in equal volumes just before using. Butyl alcohol was used for clearing. The material was imbedded in paraffin. At the beginning it was sectioned, some transversely, some longitudinally; but later all specimens were sectioned only longitudinally, 10 μ thick. The sections were stained with Flemming's triple stain.

The sections were studied critically for evidence of bud primordia and for later stages of bud development. The sites of such activity were noted, and counts, in terms of occurrence of these sites in the lower and the upper halves of the hypocotyl, were made.

In addition, the gross behavior of the nondecapitated and the decapitated hypocotyls was noted for correlation with the results of the histogenetic studies.

Observations and findings

a) GROSS BEHAVIOR OF THE HYPOCOTYL

The hypocotyledonary axis of the 1–2-day-old seedling is characterized by a bend which turns the root downward. This bend promptly takes the shape of an inverted U and then, as it straightens, pulls the seed coats and cotyledons out of the substrate. It is the first part of the seedling to emerge from the substrate. In our May plantings straightening was completed by the close of the 4th day. The rate of these events depends upon internal factors, such as the age of the seed, and upon external factors, such as temperature, moisture, and illumination.

The bend and straightening result from successive cell elongation on opposite sides of the hypocotyledonary axis. The cells on the prospectively convex side elongate first; this causes the bend and arch. Then the cells of the concave side elongate; this causes the straightening. After this has been accomplished, a phase of zonal elongation sets in. In soil- and sand-grown seedlings, this phase of zonal elongation begins at the base of the aerial part of the axis, as reported by CROOKS (5), and then proceeds progressively upward along the hypocotyl until the zone immediately below the

cotyledons is reached. While the straightening phase usually is completed in 4–5 days, the zonal-elongation phase may require 5–15 days more for completion, but duration of this phase, too, depends upon internal and external factors.

If a decapitation cut is made in the region of zonal elongation or above it, the decapitated stump keeps on elongating, the amount of elongation depending upon the amount of actual and potential elongation zones left by the cut. For example, if hypocotyls are severed on the day of emergence of the bend, at the top of the bend while it is only 1–2 mm. above the soil, the stumps straighten, swell, become greener, and increase two to five times in length during the next 5 days. After that, elongation ceases. Such stumps develop an abundance of buds. The later and the higher up the cut is made, the less the increase in length of the stump.

b) PHELLOGENIC ACTIVITY OF THE HYPOCOTYL

Generally, by the 30th to 40th days, the nondecapitated hypocotyl begins to lose its deep-green color, and its surface begins to crack and to become scaly. In the meantime, slight but general tangential stretching in part accommodates the epidermal and other cortical cells to the diameter increase of the hypocotyl. In addition, some accommodation results from radial divisions and subsequent cell enlargement, especially in the hypodermis and deeper cortical cells (fig. 7). Tangential divisions begin to appear in the epidermal cells after the 30th day. Several of these divisions may take place within the confines of the original epidermal wall. Later, tangential division proceeds to the hypodermis, the deeper cortical cells, and the pericycle. After the first tangential division, the derivatives may experience radial division (fig. 7). The walls of the phellem are not heavily suberized. A phelloderm is lacking. Cracking and sloughing begin in the lowest zone of the hypocotyl and proceed upward. In 90-day-old material, sloughing had extended into the pericycle.

The young decapitated hypocotyl becomes greener than the nondecapitated. It usually swells immediately after decapitation, especially at the upper end, largely because of increase in size of intercellular spaces. In time, the epidermis, deeper cortex, and pericycle successively become phellogenic, as in the nondecapitated hypocotyl.

c) MODE OF INITIATION OF HYPOCOTYLEDONARY BUD PRIMORDIA

BURNS and HEDDEN (4) reported that the hypocotyledonary buds of *L. usitatissimum* are of epidermal origin. BEALS (3) and CROOKS (5) have verified this report. At the onset of bud-primordium initiation, the epidermis consists of cells which are longitudinally elongated. Some of these cells are two to three times as long as they are wide, many of them are much longer, and a few are shorter. Many of the epidermal cells are more or less pointed at one or both ends. When mature, their outer wall is cutinized. At this stage the guard and accessory cells are fully differentiated. The mature epidermal cell possesses a large central vacuole. Neither the cytoplasm lining its wall nor its nucleus is very prominent. Occasionally one of these cells bears one or more cytoplasmic bridges across the vacuole.

There is no grossly detectable increase in size of the epidermal cell immediately preceding the first division leading to bud-primordium initiation (figs. 1, 2), nor is there marked increase of the cyto-

plasmic contents of the cell about to divide or of the daughter cells. Neither is the position of the nucleus in a cytoplasmic strand transversely across the central vacuole a diagnostic sign of bud-primordium initiation. In fact, the cells which are about to engage in, or just have engaged in, the first division in bud-primordium initiation do not appear very different from mature epidermal cells which divide radially in the course of diameter increase of the axis or from those which are becoming phellogenic or have just completed the first tangential division characteristic of phellogenic activity. RAUH (9) reports that, prior to the division which initiates bud-primordium formation, the cells involved become rich in cytoplasm. Neither BURNS and HEDDEN (4) nor CROOKS (5) made mention of this. Even after several additional divisions within the confines of the old cell wall, the new cells are not characterized by marked replacement of the central vacuoles with cytoplasm. There is an increase in cytoplasm, but it is peripheral in the new cells and does not become strikingly evident until the derivative cells have become numerous and quite small by repeated divisions in several planes (figs. 3, 6).

FIGS. 1–3.—Fig. 1, tangential section of an 11-day-old nondecapitated hypocotyl, showing epidermal cell with transverse divisions which initiate bud-primordium formation. Fig. 2, shallow tangential section of a 9-day-old hypocotyl, decapitated on the 5th day, showing epidermal cell, adjoining stoma, with transverse divisions which initiate bud-primordium formation. Fig. 3, shallow tangential section of a 15-day-old nondecapitated hypocotyl showing an epidermal cell with four transverse walls and a radial wall across each derivative cell. Often the radial division is preceded or followed by tangential division.

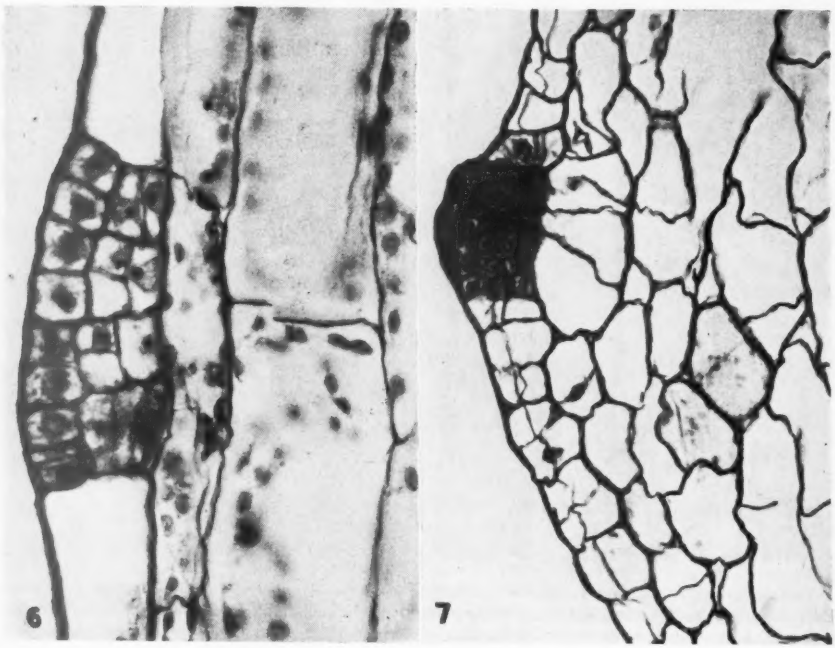
In all material studied by us, the first division in initiation of the chain of divisions involved in bud-primordium forma-

tion is transverse (figs. 1, 2), as found by Eggers in 1937 (6). Generally, but not invariably, this is followed by one or several additional transverse divisions (figs. 1, 2) within the confines of the old wall. These early transverse divisions are soon or less simultaneously, some may divide tangentially, some radially, some transversely (figs. 4, 5, 6). After repeated divisions of the original cell derivatives, the cytoplasmic content of the new cells within the original cell has increased to

FIGS. 4–5.—Fig. 4, shallow tangential sections of a 9-day-old hypocotyl decapitated on the 5th day. The primordium had its origin in two adjoining pointed cells, which divided transversely more or less simultaneously. Following these divisions the flanking epidermal cells have undergone transverse division, and the epidermal cell adjoining the stoma has undergone transverse and radial division. Fig. 5, shallow tangential section of a 9-day-old hypocotyl, decapitated on the 5th day. The epidermal cells first to divide are seen almost in surface view. The original transverse divisions were followed by uneven transverse and radial divisions of the derivatives. The flanking epidermal cells have undergone division, transverse (left), and transverse and irregular radial (right). The lowest cell to the right adjoins a stoma.

followed by tangential, or by radial, divisions (fig. 3) or by a combination of these (fig. 6). The latter is possible because the new cells formed by the first and the immediately subsequent divisions do not always behave alike as to orientation of their spindles; hence, more such an extent and the nuclei are so evident that the meristematic area is easily detected in stained material. These meristematic sites are detectable after a few divisions with considerable certainty in longitudinal sections, but in transverse sections of the hypocotyl it is possible to

detect evidence of these events with certainty only after radial and tangential walls have come in, following the transverse wall or walls. This may account for the fact that CROOKS (5) and RAUH (9) both missed the earliest stage of bud-primordium initiation and represented the two-division stage as a four-celled stage resulting from a radial and a tangential division. CROOKS did not indicate which of these divisions he thought comes first. RAUH states that the radial generally, but not invariably, comes first. There is little, if any, cell enlargement during the first stages of transverse cell division (figs. 1, 2, 3). However, increase in volume of the derivatives becomes quite evident after radial and tangential divisions have taken place. The increase is due to increase in number and volume



FIGS. 6–7.—Fig. 6, longisection of an 11-day-old hypocotyl decapitated on the 5th day. Three transverse divisions of an epidermal cell gave rise to derivative cells, which underwent two tangential divisions, with subsequent transverse and tangential division in only some of their derivatives. Many primordia in this stage also show radial division. The primordium has attained the "bulge" stage. No enlargement or divisions are evident in the hypodermal cells underlying it. In nondecapitated hypocotyls very few primordia reach this stage of development. Fig. 7, transverse section of a nondecapitated hypocotyl, 36 days old. Two epidermal cells have undergone a combination of transverse, radial, and tangential divisions and given rise to a primordium, which bulges above the surface. The cells of the hypodermis below the primordium are excessively enlarged and have divided radially. This might be only a response to tangential stretching or the beginning of the meristematic activity, which eventually may extend from the primordium to the endodermis and, in case the primordium becomes a bud, to the vascular elements of the main axis. The epidermis has become phellogenic and also shows some radial divisions. The other cortical cells have accommodated themselves to diameter increase of the axis by tangential stretching and radial division.

of nuclei, cytoplasm, and walls, and not to increase in size of vacuoles.

The subsequent stages of bud-primordium development are correctly described and figured by CROOKS (5) and RAUH (9). The epidermal cells adjoining the originally dividing one may become involved in chains of division like that of the cell first to divide (figs. 4, 5). If the events proceed further, the mass of epidermal derivatives begins to bulge above the surface of the hypocotyl (figs. 6, 7). We designate this stage the "bulge stage." Later, leaf primordia are formed which initiate the "bud" stage. The bulge is largely due to the mass of new cells formed, and not to vacuolar enlargement. The primordium, the subsequent bud, and the shoot are wholly derived from epidermal cells. The mature epidermal cells therefore are totipotent. Whether or not the derivatives of a single epidermal cell can develop a shoot we were not able to determine. In case that bud-primordium development proceeds beyond the bulge stage, the underlying hypodermal cells become meristematic, as first reported by BEALS (3) and later verified by CROOKS (5) and RAUH (9). After this, the meristematic activity extends progressively through the cortex and pericycle, until the phloem and the xylem are reached. Later, the vascular derivatives of these meristems establish connections between the vascular elements of the bud and of the axis. The hypodermal and deeper cells involved generally enlarge before they begin to divide in the course of linking the bud with the vascular tissues of the main axis. These stages are not given in this paper because they are reported and excellently illustrated in the paper by CROOKS (5).

The course of events in bud-primordium initiation and development may be arrested at any stage. If stopped immediately after the first transverse division, it is not possible to identify positively the derivatives as prospective bud-primordium initials, even if one has a full-length surface view or a shallow tangential section of the cell involved.

A paper by BAIN (2) described the same course of events for the decapitated hypocotyl of cranberry. These identical findings for plants of different families raise the question of whether the course of cytogenesis in their hypocotyledonary bud formation is normal for other plants which initiate hypocotyledonary buds in the epidermis.

d) SITE AND TIME OF INITIATION OF HYPOCOTYLEDONARY BUD PRIMORDIA

1) IN THE NONDECAPITATED HYPOCOTYL.—By the 5th day after planting or soaking of our May, 1939, material, the epidermal zone of the hypocotyl at, and immediately above, the ground line was made up of cells which either were elongating rapidly or had completed most of their longitudinal stretching. Almost invariably, among the epidermal cells of this zone in hypocotyls of this age, cells occur which are undergoing or have undergone the transverse divisions which initiate the chain of divisions that leads to formation of bud primordia. This finding was checked by examination of many other lots of nondecapitated and decapitated hypocotyls. We have never seen evidence of these divisions in 4-day-old seedlings. Initiation of this chain of divisions by epidermal cells of the normal, nondecapitated hypocotyl, therefore, begins between the 4th and the 5th days of development of the seedling. This is just at the close of the period of straightening of the hypocotyl and before the shedding of the seed coats and the enlargement of

the cotyledons. The zone in which these events occur is not determined by the soil line but by factors internal to the seedling. *Linum usitatissimum* does not form root buds.

Very often the epidermal cells adjoining the ends of the stomatal cells or cells near a stoma are the first to divide (figs. 2, 3). Short, but apparently fully elongated cells, pointed or not pointed at one or both ends, often are involved (figs. 3, 4), but division is not restricted to, or even more frequent in, these short cells. Often two adjoining short cells which occupy about the width of one of the longer epidermal cells are simultaneously the site of these divisions (fig. 4). They are easily detected in surface views of the epidermis. We have never seen a bud primordium in the upper half of a nondecapitated hypocotyl.

In the material of the May planting the largest number of bud primordia in a nondecapitated hypocotyl was found in a 15-day specimen. This hypocotyl grew in soil and bore thirty-five primordia on its lower half. Of these, thirty-two were in the early stages, and three had attained the bulge stage, but not the bud stage. An 11-day-old specimen bore fourteen bud primordia, and only one of these had attained the bulge stage. In a 7-, 6-, and 5-day-old hypocotyl, thirty-two, ten, and five bud-primordium sites, respectively, were counted. None had attained the bulge stage. Seven-, 6-, and 5-day-old hypocotyls grown in sand bore six, four, and three sites, respectively. None of these primordia had attained the bulge stage. There is great variation in the number of primordium sites in individual hypocotyls of the same age, and no readily demonstrable correlation between the number of bud primordia and the age of the hypocotyl has been found.

The oldest nondecapitated hypocotyl examined critically by us was fixed when 36 days old. It bore only six bud primordia, all restricted to the lower half. Three of these had attained the bulge stage. The hypodermal cells below these primordia had enlarged and possibly started (fig. 7) the chain of divisions incident to connecting a bud with the vascular system of the hypocotyl. Epidermal and hypodermal cells bordering the large and small primordia showed evidence of the stretching and divisions which accommodate these cells to diameter increase of the axis (fig. 7), and in the epidermis the tangential divisions of phellogenic activity were in evidence (fig. 7). While this material, like others examined by us, shows no evidence that bud primordia are isolated or sloughed off by periderm formation beneath them, we are not able to state unqualifiedly that this never occurs. Supposedly, the primordia, once formed, lie dormant in various stages of development in the nondecapitated hypocotyl.

Bud and shoot development occasionally proceeds from these primordia in nondecapitated hypocotyls of old plants, as reported by BURNS and HEDDEN (4) and as observed by us. BURNS and HEDDEN found such shoot development in 7 per cent of "uninjured" hypocotyls of plants grown in crocks which were kept moist. We noticed them in sand cultures under conditions of high nitrate nitrogen nutrition and bright sunlight. CROOKS did not record observation of such shoots. RAUH (9) stated that the perennial species of *Linum*, like many other plants which may develop subcotyledonary shoots without removal of the main axis (which he put into a class of facultative subcotyledonary shoot development), do so frequently in culture under favorable nutritive conditions but only rarely in their native habitats.

Whether the relatively few cases of development of these primordia into buds and even into shoots in the nondecapitated hypocotyl take place in absence of injury of any sort to the hypocotyl we were not able to determine. In only one young hypocotyl of the many nondecapitated ones of the May planting examined by us did we find a site which had progressed beyond the stage of epidermal involvement. In this instance the epidermal derivatives bulged above the surface of the 7-day-old hypocotyl, and the hypodermis and several layers of the cortex had become meristematic. While this hypocotyl was free of any grossly detectable wound, microscopic examination revealed a puncture, which extended into the cortex of the axis above the site of the primordium. It is possible, therefore, that some of the hypocotyledonary shoots which develop occasionally from nondecapitated hypocotyls result from wounds of the hypocotyl which are not grossly detectable.

2) IN THE DECAPITATED HYPOCOTYL.—BURNS and HEDDEN (4) reported that the seedlings of *L. usitatissimum*, if decapitated immediately below the cotyledons when 2–3 cm. high, produce buds which are not restricted to the base of the hypocotyl and range in number from one to sixty or more per hypocotyl. They also found that the age of the hypocotyl at time of decapitation is a factor in bud formation both as to its frequency and as to its site. CROOKS (5) reported that seedlings decapitated before they are 10 days old produce buds in 6–8 days and that 60 per cent of plants severed below the cotyledons when 60 days old died without producing buds. He found that all hypocotyls decapitated when not more than 10 days old produce five to twenty buds on the remaining part of the hypocotyl, even when the cut was made as low as a few millimeters above the soil line. EGGERS (6, 7) corroborated and enlarged upon these findings and found that, in general, decapitation on the 10th day after emergence of the seedling results in maximum survival of plants and maximum bud production under the various conditions of nutrition tested at different times of the year. In the course of our studies we found that decapitation at any level and at any age up to 60 days, and occasionally even more, could be followed by grossly detectable bud development. With increasing age and severe external conditions, such as high temperature, intense light, low humidity, and poor ventilation, an increasing number of hypocotyls die with or without development of buds and with a decrease in the number of buds developed per plant.

CROOKS (5) showed that the observation made by BURNS and HEDDEN (4), that not all buds start grossly detectable development at the same time, is due to the fact that, while buds on one part of the hypocotyl are large enough to show small leaves, many others are in earlier stages of development. He found that after eight to ten buds begin to show small leaves, one bud usually outgrows all the others. This bud may be at any level in relation to the others, and it is not necessarily the first to have made its gross appearance. Occasionally, more than one bud continues development, but, in time, one gets the ascendancy. Our observations are in agreement with these findings by CROOKS.

BURNS and HEDDEN (4) reported that, following decapitation of the hypocotyl, its epidermal cells become rich in chloroplasts. We did not find this to be the case. CROOKS reported that the swelling of the hypocotyl noted by BURNS and HEDDEN following decapitation is due to formation of large intercellular spaces. We

were able to corroborate that finding. CROOKS reported that the hypocotyledonary bud is initiated by division of an epidermal cell and that this division is quickly followed by a second at right angles to it, producing a four-celled stage, with a radial and a tangential wall. RAUH (9) reported the same for *L. usitatissimum* and for *L. tenuifolium*. We found that the mode of bud-primordium initiation in the decapitated hypocotyl is the same as in the nondecapitated hypocotyl, i.e., it begins with a transverse division of an epidermal cell which is elongating rapidly or, more generally, has completed its elongation (fig. 2). Here, too, the cells which divide transversely are not necessarily the most elongated ones. In fact, many of them are very short. As in nondecapitated hypocotyls, epidermal cells adjoining the ends of stomatal cells or near the stomata seem to be the most frequent sites of these divisions (fig. 2).

While no epidermal cells in the upper half of the nondecapitated hypocotyl initiate bud primordia, the cells in this region of the decapitated hypocotyl initiate bud primordia in great abundance.

In our May, 1939, material, decapitated immediately below the cotyledons when the seedlings were 5 days old, a hypocotyl 2 days after decapitation (7-day-old material) bore twenty-one sites of bud-primordium initiation in its lower half. These primordia were in about the same stage of development as those in a nondecapitated hypocotyl of the same age. Another specimen grown in sand bore sixteen sites, all restricted to the lower half of the hypocotyl. None of the primordia had attained the bulge stage.

By the 4th day after decapitation, striking differences were evident between nondecapitated and decapitated hypocotyls (all 9 days old). As pointed out before, none of the nondecapitated hypocotyls bore bud-primordium sites in the upper half of the hypocotyl, and none of the primordia had advanced past the bulge stage. In contrast, a hypocotyl grown in soil, 9 days old and 4 days after decapitation, bore fourteen sites in its upper half and thirty-five in its lower half. None of the primordia of the upper half had attained the bulge stage, but one in the lower half had. A specimen of the same age but grown in sand bore thirty sites in its upper half and twenty-three in its lower half. Eight of the former sites bore primordia in the bulge stage, and one bore a bud stage. None of the primordia of the lower half had attained the bulge stage. The findings for hypocotyls 10 days old (6 days after decapitation) are of the same order except that more sites bore advanced stages. The hypocotyl grown in soil bore nine sites in its upper half and thirty-nine in its lower half. In three of the former the primordia were in the bulge stage and in two, in the bud stage. In twelve of the sites in the lower half, the primordia had reached the bulge stage and in one, the bud stage. A hypocotyl of this age grown in sand bore thirteen sites in its upper half and twenty in its lower half. In two of the former, the primordia were in the bulge stage and in four, in the bud stage. In two sites in the lower half, the primordia had reached the bulge stage and in two, the bud stage. No sample of a hypocotyl 15 days old (10 days after decapitation) grown in sand was sectioned. The soil-grown sample bore fourteen sites in its upper half and sixteen in its lower half. In two sites in the upper half the primordia had reached the bulge stage; five had reached the bud stage; and one had become a shoot, 2 mm. long. It was located near the top

of the decapitated hypocotyl. In two sites of the lower half, the primordium had reached the bulge stage.

Examination of many other nondecapitated and decapitated hypocotyls indicates that, on the average, the decapitated hypocotyl bears more bud primordia in its lower half than the nondecapitated. However, variation in the number of bud primordia and buds per hypocotyl is so large that an extensive statistical study would have to be undertaken to determine whether or not decapitation increases the number of primordia in the lower half of the decapitated hypocotyl. Occasionally, one finds a decapitated hypocotyl which does not bear any primordia in its upper half.

In order to determine whether or not the absence of buds and shoots from the upper half of hypocotyls decapitated late is due to noninitiation of primordia by this region or to nondevelopment of primordia present at time of decapitation, we examined the hypocotyls of plants which had been decapitated on the 36th day and collected and fixed on the 46th day. As in the nondecapitated hypocotyls of this lot recorded above, no primordia were found in the upper half of the axis, and fifteen were found in the lower half. This corroborates our general finding that the epidermal cells of the upper half of the hypocotyl, and probably of the lower half, in time lose their capacity to initiate bud primordia but that the capacity to develop the bud primordia present into buds and shoots may be retained up to the 60th day, and even longer.

During the 10 days following decapitation of this 36-day-old plant, one bud primordium had progressed to the stage of leaf-primordium formation and the hypodermis and three additional layers of the cortex had become meristematic in the manner described by CROOKS (5). This is a slow rate of development compared with that of primordia of hypocotyls decapitated between the 5th and the 10th days. As noted before, when hypocotyls are decapitated later than the 10th day, the chances of their survival are greatly reduced. If permitted to grow, this bud in the 36-day-old material probably would have established connections with the vascular tissues and grown into a shoot, provided the hypocotyl did not die. As in the material collected on the 36th day, there was no evidence that the increased phellogenic activity from the 36th to the 46th days had extended below the sites of even the youngest bud primordia. We have found no instance of phellogenic origin of hypocotyledonary buds.

Discussion

Some of the current interpretations of the hypocotyledonary bud situation in *L. usitatissimum* are inadequate because they do not consider the fact that epidermal cells of the lower half of the normal, nondecapitated hypocotyl undergo the first stages of bud-primordium formation and that these primordia occasionally develop into buds and shoots. BURNS and HEDDEN (4) attempted to explain their observations that a nondecapitated hypocotyl occasionally develops a shoot at its base and that decapitated hypocotyls, especially those decapitated when old, frequently develop shoots only at the base, by assuming that the base of the hypocotyl is "especially predisposed" to bud formation and that it retains its ability to form buds longer than the rest of the hypocotyl.

In his studies of substitute shoot and root formation (*Ersatzbildung*) by vari-

ous species of *Linum*, NEUBERT (8) found it necessary to distinguish between (1) replacement of the lost member in a similar site (*Wiederbildung*); (2) *de novo* formation of a member at the site of loss (*Neubildung*); and (3) development of a dormant member which replaces the lost member (*Neuentfaltung*). Not being aware that some of the hypocotyledonary shoots of *L. usitatissimum* are the result of development of a dormant member, he considered all the hypocotyledonary shoots of this plant to be examples of replacement of a lost member in a similar site.

RAUH (9) recognized three types of subcotyledonary shoot formation, which he designated (1) obligate, (2) facultative, and (3) regenerative. He used the plant's dependence or nondependence for survival on development of subcotyledonary shoots as a criterion to define and differentiate the obligate and facultative types. In defining and differentiating the regenerative type, he did not use this criterion but adopted the ability of the plant to form buds after removal of the main axis as the criterion. Had he not shifted criteria, he would have been able to consider the regenerative type a subtype of the facultative type. RAUH ignored BURNS and HEDDEN's report that *L. usitatissimum* occasionally produces subcotyledonary buds and shoots without decapitation of the hypocotyl, and he was not aware of the presence of bud primordia in the lower part of the normal hypocotyl of this plant. Accordingly, he classified its hypocotyledonary shoots as regenerative.

The system of classification formulated and the criteria adopted by RAUH can be employed more logically and consistently than he has done. This may be accomplished by retention of the obligate and facultative types and by division of the latter into at least two subtypes. The first subtype may be defined so as to comprise those subcotyledonary shoots which develop without removal of the main axis, and the second so as to comprise those which develop after removal of the main axis. Subtype 2 needs division into group A, comprising those shoots which develop from buds (primordia) laid down before removal of the axis, and group B, comprising those shoots which develop from buds (primordia) laid down after removal of the axis. According to this modified system, some of the hypocotyledonary shoots of *L. usitatissimum* belong to the first subtype, some to the second, and, of the latter, some belong to group A, some to group B. Reinvestigation of the other plants with subcotyledonary shoots which have been classified by RAUH may reveal that in them, too, the subcotyledonary shoot situation is more complex than appears superficially.

Our findings indicate that the epidermal cells of the hypocotyl must be in a definite stage of development for initiation of the chain of divisions which characterizes bud-primordium formation. This stage apparently is realized while the epidermal cells are in the final stages of elongation and, for a time, after this elongation has ceased. The epidermal cells which are sites of the first transverse divisions of bud-primordium formation do not experience greater enlargement prior to the first division than do their neighbors which do not divide, nor do they show marked increase in cytoplasmic content. Our findings also indicate that initiation of this chain of divisions by cells in the proper stage of development may begin in the presence of the cotyledons and epicotyl on the 5th day in the development of the seedling but that after a short time it is inhibited,

so that in intact seedlings bud primordia are found only in the lower half of the hypocotyl, whereas in the decapitated young hypocotyl they occur at all levels of its remainder.

Further development of the early stages of bud primordia also is affected by the cotyledons and epicotyl, so that, except occasionally, it does not progress beyond the stage of epidermal involvement and stops short of leaf-primordium formation. In intact plants the primordium only rarely develops into a bud, and the bud develops into a shoot even more rarely.

These inhibitions of the capacity to begin and to continue the chain of divisions which characterize bud-primordium initiation and development are not instances of a generalized inhibition of meristematic capability of epidermal cells of the hypocotyl. At the time of these specific inhibitions of bud-primordium initiation and development, these cells still are capable of radial division and even more of the tangential divisions which characterize phellogenic activity.

Summary

1. Transverse division of an epidermal cell is the first identifiable stage in initiation of a hypocotyledonary bud primordium in *L. usitatissimum* L.

2. Generally, a second transverse division in one or both of the daughter cells, but not infrequently either a radial or a tangential division, is the second stage.

3. These divisions are not preceded by grossly detectable increase in size of the cells involved, nor is there marked increase of the cytoplasmic contents of these cells prior to or immediately following these divisions.

4. These events begin in epidermal cells of the lower half of the hypocotyl between the 4th and 5th days of the developing seedling. Development of primordia of the lower half of the hypocotyl into buds, and of these buds into shoots, rarely occurs in the normal, non-decapitated hypotocyl.

5. Upon decapitation of the hypocotyl, one to many of the bud primordia of the lower half of the hypocotyl may develop into buds, and one or several of these may develop into shoots.

6. The epidermal cells of the upper half of the hypocotyl also are capable of initiating bud-primordium formation but do not do so unless the hypocotyl has been decapitated. Following decapitation between the 3d and 15th days after emergence of the seedling, a great number of primordia may be initiated by these epidermal cells. Many of these primordia may develop into buds, and one (occasionally several) of these buds may develop into a shoot. After a shoot from either the lower or the upper half of the hypocotyl has established dominance, the development of the other shoots ceases or proceeds very slowly.

7. The epidermal cells apparently must be in a definite stage of development for initiation of the chain of divisions which characterizes bud-primordium formation. This stage apparently is realized while or after the epidermal cells are in the final stage of elongation and persists for a time after this elongation has ceased. It is first attained in the zone of general elongation in the lower half of the hypocotyl and proceeds upward as this zone moves up along the hypocotyl.

8. Presence of the cotyledons and epicotyl does not inhibit initiation of bud primordia in the lower half of the hypocotyl but does inhibit it in the upper half. Generally, the presence of these organs and, later, of the epicotyl alone inhibits

development of the bud primordia of the lower half of the hypocotyl into buds and of the occasional bud into a shoot.

9. Generally, if hypocotyls are decapitated after the 20th–30th days after emergence, no bud primordia are formed in the upper half of the hypocotyl, but some of the primordia present in the lower half may develop into buds, and, of these, one or rarely two may develop into a shoot.

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THE RELATION OF PHOTOPERIOD TO THE BORON REQUIREMENT OF PLANTS[1]

ROBERT MACVICAR AND B. ESTHER STRUCKMEYER

Introduction

In the course of experiments on the boron nutrition of plants, it was observed that plants responding to short photoperiod exhibited diminished response to boron deprivation. WARINGTON (9) had noted in *Vicia faba* and other plants that there was a delay in producing symptoms of boron deficiency during shortened days and had attributed such effects to differential rates of growth. DUFRENOY (3) had also observed, in a study of the effect of boron on the development of the root-nodule system in the same plant, that artificially extending the photoperiod during February and March hastened the development of symptoms of boron deficiency. Since the present study was undertaken, SKOK (7) has also reported that, under short photoperiods, the symptoms of boron deficiency in the radish were less marked.

Methods

Soybeans (var. Biloxi and Manchu no. 3) were selected because of their known response to photoperiod (2) and because previous work had shown them to have a reasonably high boron requirement when grown in long days. Seeds were disinfected with $CaOCl_2$ and germinated in silica sand. After the development of the

[1] Published with the approval of the Director of the Agricultural Experiment Station.

first true leaves, the seedlings were transferred to leached silica sand in varnished, 10-inch clay pots. Five plants were planted in each pot, and there were four replications of each treatment. Approximately 500 ml. of the following nutrient solution was added four times weekly:

	Experiment 1	Experiment 2
K_2HPO_4.....	0.0012 Molar	0.0012 Molar
KH_2PO_4.....	.0018	.0018
$Ca(NO_3)_2$....	.0084	.0042
$CaCl_2$.....	.0011	.0011
$Mg(NO_3)_2$...	.0042	.0021
$MgSO_4$.....	.0011	.0021
NH_4NO_3.....	0.0042	0 0021

Boron was added at a 0.5-p.p.m. level. Other micronutrient elements were those recommended by Hoagland and Arnon (4). All pots were thoroughly leached with distilled water at least once weekly to remove any unbalanced solution.

All plants were grown under normal greenhouse conditions of light intensity, humidity, and temperature. During the summer months the houses were whitewashed. In experiment 1, plants grown in normal day length were placed in a more exposed house having a somewhat higher sunlight intensity, 1400 foot candles as compared with 1000 foot candles in those houses under controlled lengths of photoperiod. The short photoperiod was varied by manually operated screens. Incandescent light bulbs, with an automatically controlled time switch, provided extended length of photoperiod from near sunset until midnight.

Samples were customarily taken in the early morning before 9:00 A.M. to minimize variations in water content. After being removed from the pot, the tops and roots were separated, the roots were freed of sand and blotted dry between sheets of absorbent paper, and the parts were weighed and immediately transferred to drying ovens. After remaining in these ovens under a constant stream of air at 60° C. until reaching a constant weight, they were re-weighed, ground to a fine powder in a Wiley mill, and stored for analysis.

Samples for microscopic examination were taken at the fourth internode from the tip and fixed in formalin-aceto-alcohol. These were dehydrated in *n*-butyl alcohol and imbedded in paraffin. The stems were cut 12 μ in thickness on a sliding microtome. The sections were stained with iron-alum-hematoxylin and safranin.

Ash was determined on the 60° air-dry samples by combustion in a thermostatically controlled muffle furnace at 500° C. Boron was determined by the quinalizarin procedure of Berger and Truog (1), as modified in this laboratory (6).

Results

Experiment 1.—Soybean seedlings (Manchu no. 3) were transferred to pots on April 17, 1941, and on April 19, 1941, and were placed in differential photoperiods of 9 and 17–18 hours, respectively. By May 10, boron-deficient plants in long days were showing restricted growth, but there was no detectable difference between plus and minus boron cultures in short days. A month after being placed in different photoperiods, boron-deficient plants in long day had developed typical symptoms, including fragility of the leaves and petioles, necrosis of apical meristematic tissue, and reduced secondary-root formation. Boron-deficient plants in short day, however, continued to develop in a nearly normal manner for plants which fruit in this photoperiod. Blossom primordia had appeared in all treatments in short and normal days; long-day plants continued to remain vegetative throughout the en-

tire length of the experiment. At the time of harvesting (June 4, 1941), boron-deficiency symptoms were very severe in long-day cultures and moderately severe in normal day length, while plants in short day appeared essentially normal. Data with reference to growth, per cent dry matter, top/root factor, and ash and boron content are presented in table 1. Figure 1 shows the appearance of the plants on May 21.

EXPERIMENT 2.—This experiment was essentially a duplicate of the previous one, except that the short-day variety, Biloxi, was used. Plants were transferred to differential day lengths on October 21,

TABLE 1

EXPERIMENT 1: EFFECT OF PHOTOPERIOD ON GROWTH AND BORON CONTENT OF NORMAL AND BORON-DEFICIENT MANCHU SOYBEANS

TREATMENT	AVERAGE DRY WEIGHT OF TOPS (GM.)	DRY MATTER (PER CENT)	TOP/ROOT	LEAF TISSUE	
				Ash*	Boron†
	5-21-41 sampling				
Long day plus boron	1.24	13.7	4.2	12.8	63.8
Long day minus boron	0.56	14.3	3.7	11.6	13.7
Normal day plus boron	1.16	20.7	2.0	8.6	46.8
Normal day minus boron	1.25	21.4	2.7	8.7	16.2
Short day plus boron	0.59	13.7	3.5	13.4	56.8
Short day minus boron	0.66	15.1	3.7	12.5	21.1
	6-4-41 sampling				
Long day plus boron	3.36	17.5	6.7	11.6	61.2
Long day minus boron	1.18	17.9	4.0	11.3	16.8
Normal day plus boron	3.10	22.5	2.9	11.8	88.7
Normal day minus boron	2.80	27.2	2.3	8.8	14.6
Short day plus boron	1.69	20.9	4.9	11.3	48.5
Short day minus boron	1.92	25.3	4.1	11.7	14.5
	6-24-41 sampling				
Long day plus boron	4.76	23.2	7.8	10.4	58.9
Long day minus boron	1.01	20.6	4.8	13.6	21.5
Normal day plus boron	9.90	21.4	6.4	9.9	62.1
Normal day minus boron	4.83	26.4	4.0	10.3	19.8
Short day plus boron	1.15	25.0	5.9	12.4	71.0
Short day minus boron	1.83	26.6	5.6	11.0	23.8

* In per cent on a moisture-free basis.
† In micrograms per gram of dry tissue.

1941. A month later, initial symptoms of boron deficiency had appeared in cultures in long photoperiod. By December 23 the typical fragility of the leaves, petioles, and upper internodes was pronounced; necrosis of meristematic tissue had occurred (although somewhat less extensively than in the Manchu variety); and secondary root development was restricted. Plants grown in short day produced fruits, regardless of the level of boron supplied. Data concerning the growth of these plants are presented in table 2.

Anatomical observations

The anatomical structure of soybean plants (Manchu no. 3) grown with and without boron in long days was quite different (figs. 2, 3). The normal stem was in the budding stage when sampled, and it exhibited the anatomical characteristics typical of budding stems, namely, a thickening of the walls of the vascular tissue and a decrease in cambial activity (8) (figs. 2, 6). The plants grown without boron remained in the vegetative state and showed an unusual type of stem anatomy. The phloem region was about four times the diameter of the normal stem (figs. 3, 8). The most conspicuous difference was the increased number and size of the phloem cells and ray cells resulting from abnormal cambial activity and some proliferation. Numerous sieve tubes and companion cells had been formed, and considerable necrosis had occurred in these regions. The ray cells especially had stretched in a radial direction and increased in size.

There was also a greater number of xylem elements in the boron-deficient stem, xylem parenchyma being formed before the cambium became inactive (fig. 8). Cells in the region of the pericycle showed considerable tangential stretching, radial division, and necrosis. The fiber cap had become separated into groups of fibers by internal pressure of the enlarging cells. The fibers themselves remained unchanged. There was an increase in cell size and some tangential division in the endodermis. The collenchyma cells of the cortex were much larger and thinner walled in the stems of plants grown without boron. The area of the pith region was somewhat less in the stems of these abnormal plants.

Fig. 1.—Plants of soybean (Manchu no. 3), grown in short and long days. *A*, plant grown in long photoperiod with boron; *B*, plant grown in long photoperiod without boron; *C*, plant grown in short day with boron; *D*, plant grown in short day without boron; photographed May 21, 1941.

The anatomy of the stems of plants grown with and without boron but in short days was not greatly different. Plants grown with boron had fruits and showed the characteristic anatomy of fruiting stems, such as thickening of the walls of the vascular tissue and absence of cambial activity (fig. 4). Plants grown without boron were in the blossoming state. The effects of boron deficiency were apparent in the stem (figs. 5, 7) but not so conspicuously as in plants grown

in long days (figs. 3, 8). The phloem parenchyma cells and ray cells showed some enlargement, but it was much less apparent than in plants grown in long photoperiod. Sieve tubes and companion cells were not greatly affected, and necrosis was not present. There was some tangential division in the endodermal cells, the fibers remained normal, and the cortical cells were larger than those in the normal stem (fig. 7). The great increase in number of cells due to cambial activity and proliferation in plants grown in long days was markedly less in plants grown in short days but also without boron.

TABLE 2

EXPERIMENT 2: EFFECT OF PHOTOPERIOD ON GROWTH AND BORON CONTENT OF NORMAL AND BORON-DEFICIENT BILOXI SOYBEANS

Treatment	Average dry weight of tops (gm.)	Dry matter (per cent)	Top/root
	11-24-41 sampling		
Long day plus boron	1.39	13.8	6.7
Long day minus boron	1.10	12.8	5.7
Short day plus boron	0.98	14.8	4.6
Short day minus boron	0.93	14.8	4.9
	12-23-41 sampling		
Long day plus boron	3.53	15.6	5.0
Long day minus boron	2.22	10.9	4.9
Short day plus boron	1.75	21.6	5.0
Short day minus boron	2.01	22.8	4.5

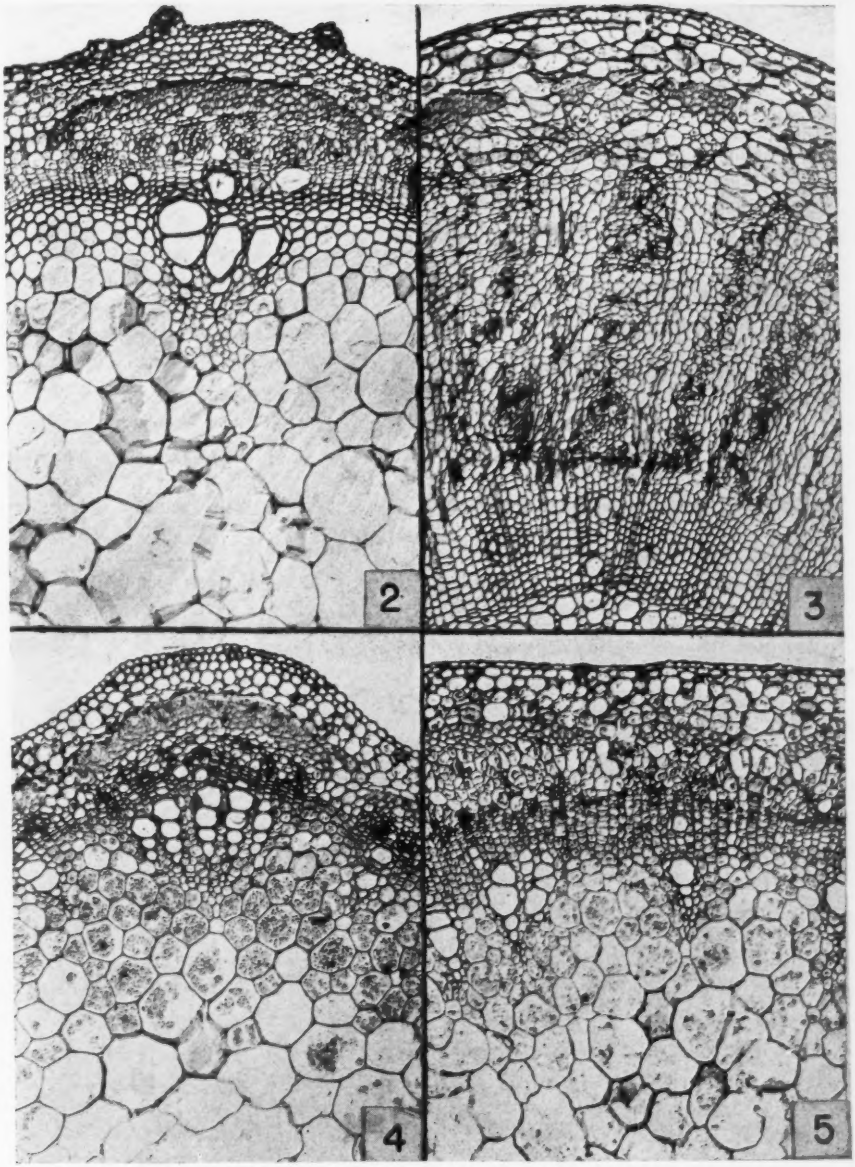
Discussion

An examination of the data presented in table 1, together with the gross appearance of the plants (fig. 1) and the more detailed anatomical structure of the stem (figs. 2–5), reveals that the symptoms of boron deficiency were far more severe in long photoperiods than in short day lengths, which induce fruit formation. This observation was confirmed with the Biloxi variety, although the differences in gross weight of tissue produced were less marked.

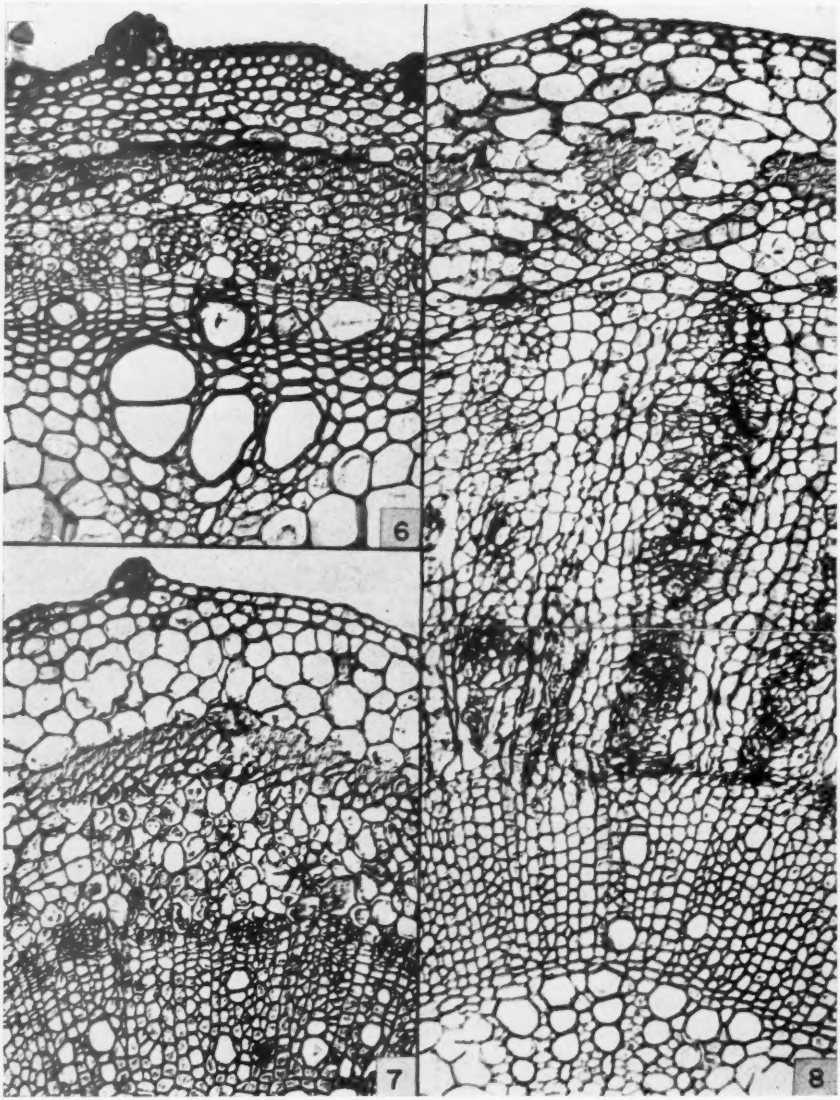
It will be noted that the severity of symptoms in Manchu was not correlated with the boron content of the tissue. Thus the boron content of the leaf of boron-deficient plants, in long and short days at the end of experiment 1, was found to be 21.5 and 23.8 μg per gram, respectively. In one group of plants, this level was adequate for essentially normal functioning; in the other, severe symptoms of deprivation resulted. The difference in boron content, therefore, was eliminated, and a reduction in boron requirement appeared to have occurred.

In boron-deficient plants in long days (fig. 8) there was an abnormal cambial activity, as evidenced by an increased amount of phloem, ray, and xylem tissue. In short photoperiod, boron-deficient plants did not exhibit such marked cambial activity. The question arises as to whether the reduced cambial activity produced by the shortened photoperiod (fig. 7), which hastened blossom induction, was responsible for the reduced need for boron. The development of the abnormal cambium in boron-deficient plants of cabbage also had been observed by JOLIVETTE and WALKER (5).

This relationship between the boron requirement and photoperiod re-emphasizes the importance of controlling the environmental conditions in studies involving the metabolism of plants. Experimental studies dealing with boron should be conducted under similar photoperiods. In attempting to produce boron



FIGS. 2–5.—Cross-section of fourth internodes of soybean (Manchu no. 3). Fig. 2, early-blossoming plant with boron in long photoperiod. Fig. 3, plant without boron in long photoperiod showing increased phloem, ray, and xylem tissues from cambial activity and proliferation. Necrotic areas also apparent. Fig. 4, fruiting plant in short photoperiod; the inactive cambium and thick-walled vascular tissue are characteristic of fruiting stems. Fig. 5, short photoperiod without boron. Compared to figure 3, only slight increase in phloem and ray tissue.



FIGS. 6–8.—Cross-section of soybean plants (Manchu no. 3). Higher magnification of figures 2, 3, and 5. Fig. 6, normal structure of plants grown with boron and long photoperiod. Fig. 7, relatively slight distortion from lack of boron in short days. Fig. 8, abnormal structure resulting from lack of boron in long days.

deficiency in plants exhibiting photoperiodic sensitivity, moreover, it would appear desirable to use long-day lengths.

Summary

1. The intensity of boron-deficiency symptoms in soybeans has been found to be much more severe in long than in short days.

2. The reduction was due to a diminished boron requirement rather than to changes in the absorption of boron. The boron content of plants exhibiting severe symptoms of deprivation (long photoperiod) and plants showing no gross evidence of deficiency (short photoperiod) was essentially the same.

3. Histological examination of the stems confirmed this observation. There was little cellular disorganization in minus-boron plants produced in short photoperiod. Boron-deficient plants in long photoperiods, however, exhibited abnormalities. There was a marked increase in vascular tissue resulting from abnormal cambial activity. Necrotic areas in the region of the phloem were conspicuous.

The authors are indeed grateful to Professor R. H. Roberts and the late Professor W. E. Tottingham for helpful advice and criticism during the course of this investigation.

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RADIAL GROWTH OF TREES AT DIFFERENT ALTITUDES

R. F. DAUBENMIRE

Introduction

Through the series of vertically stratified climates on the slopes of the Rocky Mountains, drought becomes an environmental factor of increasing importance in a downward direction, while low summer temperatures play an ever more critical role upward. In the light of existing information, these aspects of climate appear to set the limits of vertical distribution of most of the tree species (1). The present investigation is an attempt to ascertain the extent to which this climatic gradient affects the course of radial growth in certain native coniferous trees growing near their upper and lower altitudinal limits.

The study was conducted at Thatuna Ridge, the summit of which is located 12 km. northeast of the town of Moscow, Idaho. Because this ridge is oriented in an east-west direction and rises over 600 meters above the adjacent basal plain, it was possible to compare trees growing at low altitudes on the south slope with others located at high altitudes on the north slope and to obtain wide differences in climate. Because of the lateness of the opening of roads in spring and their impassibility after the rainy season begins in autumn, the study included only the 5-month period between April 19 and October 17, 1942.

Four trees were studied at each station, and a low- and a high-altitude station were established for each of the following species: *Pinus ponderosa* Dougl., *Abies grandis* Lindl., *Larix occidentalis* Nutt., and *Thuja plicata* Donn. The REINEKE dendrometer (3) was used in this investigation; and, although the apparatus proved to have an inherent error of at least ±0.002 inch, the use of averages based on four individuals overcame most of the errors in measurement. Another source of unavoidable error was the fact that the trees were measured at different times of day; and, consequently, diurnal fluctuations in radius, which are due to changes in the degree of tissue hydration, affected the measurements. This error, however, was reduced to insignificance by the facts that measurements were made only at intervals of a week or more and so were submerged by radial changes of greater magnitude and that the measurements of all trees under comparison were taken within a few hours, so that errors were approximately equal. The smoothness of the curves obtained and the fact that most radial changes were correlated with weather changes provide evidence that neither of the above sources of error can account for any of the conclusions.

The most relevant weather data available for the summer of 1942 were recorded at a co-operative station of the United States Weather Bureau located at Moscow, which is situated on the prairie-covered basal plain below the altitude of the lowest trees studied. It is believed that these data, which are summarized in figure 1, are sufficiently indicative of weather trends on the ridge to be very useful in interpreting observations.

In figure 1 is shown the quantity of water falling at each precipitation, when this amount exceeded 2.54 mm. During the rainy season precipitation undoubtedly increases with altitude on the ridge, but a series of five rain gauges maintained by the writer from July 17 to September 20, 1942, showed that there was no altitudinal gradient on the ridge during that period. In accordance with the normal

character of the climate, rainfall practically ceased in early summer, so that the warm season was also a season of cumulative drought. The significance of this dry season is to be reckoned in terms of high transpiration stress, to which at low altitudes is added the effect of desiccation of those soil horizons which contain the upper parts of the root systems.

Weekly means of the daily maximal and minimal temperatures, and weekly evaporation from a free water surface are shown in figure 1. Too much weight cannot be given the last statistic, for the Weather Bureau station is surrounded by lawns that are irrigated with sprinklers, and the latter are sometimes set so close to the instruments as seriously to affect the records.

Pinus ponderosa

Because of its relatively low altitudinal distribution, *P. ponderosa* is not found at high altitude on the north side of Thatuna Ridge, and for the upper group of trees a station in the arborvitae-hemlock zone[1] at an altitude of 1065 meters on the south slope was used. The lower station was in the ponderosa pine zone on the same slope, but at an altitude of 838 meters.

At both stations rapid radial growth was in progress by April 24, and, from the steepness of the curve (fig. 2), it may be estimated that growth had begun by at least mid-April. Until late May, the rate of growth was distinctly less at the higher station, presumably because of the lower temperature; but, starting in late May, the growth rate declined at the lower station and increased above. In early July a period of above-average heat and dryness (fig. 1) completely arrested growth at the lower station but had practically no effect above. There followed a week of moderated temperature with showers, and, in response to this change, the normal rate of growth was resumed at the lower station and slightly increased at the upper. Immediately following this last series of showers, maximal temperatures again rose to high levels and the long dry season began.

During the latter half of summer the trees at the lower station suffered considerable shrinkage. This was progressive

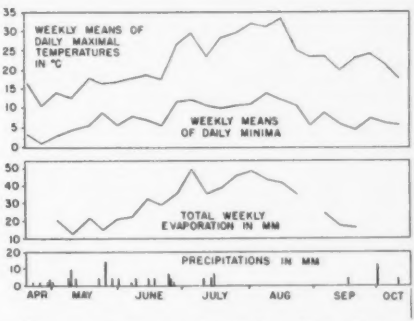
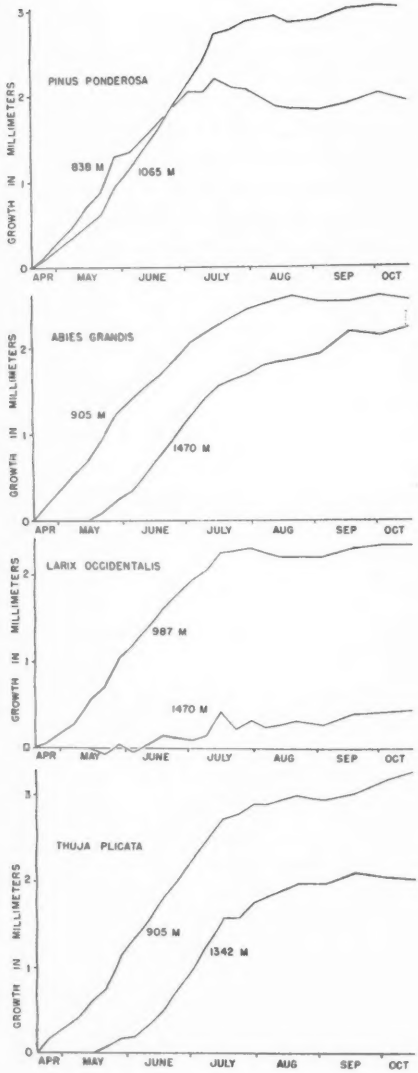


Fig. 1.—Summary of weather data taken at Moscow, Idaho, from April 19 to October 17, 1942.

until early September, when a period of cool weather, during which a small shower fell at Moscow, allowed recuperation to begin. Assuming that the evaporation data for Moscow are correct, this rain did not have an appreciable effect upon the rate of evaporation, which had already declined strongly in accordance with reduced temperatures (fig. 1). Either rainfall was considerably greater on the ridge and, in consequence, definitely reduced evaporation there, or the shower benefited the plants in some way other than through increasing relative humidity. The rain of October 1 also stimulated enlargement, but the effect was only temporary, and, during the rela-

[1] The four major forest zones of northern Idaho are, in order of increasing altitude, ponderosa pine zone, Douglas fir zone, arborvitae-hemlock zone, and spruce-fir zone. The forests of these zones have been briefly characterized elsewhere (1).



FIGS. 2–5.—Fig. 2. radial changes in two groups of trees of *Pinus ponderosa* growing at different altitudes. Fig. 3, same for *Abies grandis*. Fig. 4, same for *Larix occidentalis*. Fig. 5, same for *Thuja plicata*.

tively dry period that followed, dehydration once again set in.

At the upper station, growth continued a month later in the summer, and shrinkage was of relatively minor extent and duration. Apparently, the more favorable soil-moisture conditions there enabled the trees to take advantage of the highly efficient midsummer temperatures for a longer period, and then subsequently allowed them to retain their diametral gains. The greater increment at the upper station is in accord with the fact that *P. ponderosa* normally grows to larger size at altitudes higher than those at which it is the sole dominant of the forest.

ABIES GRANDIS

Owing to the condition of the road, the upper station for *Abies* could not be visited until May 15. From this date until June 5 the trees at the upper station grew more slowly than those at the lower (fig. 3). Late-summer shrinkage was slight at the lower station, but no shrinkage was observed above that could be definitely attributed to drought. The greater net growth at low altitude is probably attributable to the fact that temperatures were more favorable for growth, and at the same time the trees were at an elevation high enough that the water balance was not an important limiting factor.

LARIX OCCIDENTALIS

The two stations for *L. occidentalis* were essentially identical with those for *Abies*, but there was much greater difference between the two groups of individuals in *Larix* (fig. 4). In this species the trees at high altitude made very slow growth throughout most of the period of observation, but the radii were subject to alternate expansion and contraction

to a degree not observed in any other group of trees. Likewise, at the lower station the behavior of *Larix* was in marked contrast with *Abies* growing there. In *Larix*, shrinkage began about 3 weeks earlier and eventually became more pronounced; but this species also responded more quickly to the mid-September shower.

Thuja plicata

In common with the other species, the trees of *Thuja* grew more slowly at the higher station in spring and early summer (fig. 5). At the lower station there were two distinct growing seasons, which were interrupted by a period of quiescence that included slight shrinkage in late August. The second growing season began with the first showers of the rainy season and lasted until at least October 17. At high altitude, growth was essentially complete by mid-September, and the dry late-summer weather had less effect upon the radii.

Discussion

In all instances the trees at low altitude on the south slope of Thatuna Ridge were growing by April 19, 1942, and those at high altitudes on the north slope were growing by mid-May. Although the observations did not begin early enough to ascertain whether or not the dates of beginning of growth differed among the habitats, the data consistently show that the growth rates in spring and early summer are more rapid, the lower the elevation. In *P. ponderosa* this difference was pronounced, with a vertical distance of only 227 meters.

The cumulative effects of dry summer weather were felt at all altitudes on Thatuna Ridge, but at low altitude the effect was always earlier and more pronounced. In all four species the individuals at the lower stations shrank in late summer, and shrinkage was observed in *Pinus* and *Larix* at the upper stations as well.

Shrinkage in *Pinus* at low altitude was so severe that by October 17 the trees had not yet recovered completely from the long period of shrinkage which they had undergone subsequent to the maximum radius observed on July 17. Also, judging from the shapes of the curves, the radii of most, if not all, of the other groups of trees probably increased somewhat after the observations were terminated on October 17.

Data to be presented in detail elsewhere show that soils become exhausted of growth water to a considerable depth in the ponderosa pine zone, where the lowest group of pines was located; but in the arborvitae-hemlock and spruce-fir zones, in which all other groups of trees were situated, soils remain moist throughout the year. This seems to provide an explanation for the fact that, except in *Pinus*, the trees at their lower stations grew faster. Unless moisture is limiting, the lower the altitude, the greater the growth, on account of the higher temperature efficiency.

A feature of special interest in the data is the considerable difference in behavior observed between the two stands of *Pinus*, which were separated by only a little over 200 meters in altitude. As indicated above, the upper group of trees is situated where soils are favorably moist all summer. Undoubtedly, this difference in soil-moisture relations between the two habitats is far more important than the slight difference in temperature that is indicated by altitude. Therefore, it becomes apparent that the location of trees with respect to zones may be a much more significant guide to the type of radial behavior to be expected than are

strictly nonbiological data, such as elevation above sea-level.

The only previous study of radial behavior of trees growing at different altitudes in the same region appears to be that of FOWELLS (2), who studied *P. ponderosa* growing at altitudes ranging from 550 to 1830 meters in the Sierra Nevada Mountains of California. There is good agreement between the conclusions of that study and the present investigation, in that growth at low altitudes in California is likewise interrupted by a considerable period of shrinkage in late summer and that in April and May the rate of growth decreases upslope. Furthermore, the phenology of diametral growth is essentially identical in the two regions.

Periodic measurements of trees to determine their annual increment should always be made after the species has completed its seasonal growth. The present study reveals considerable difference among the species as to the earliest date when their growth can be considered essentially complete in northern Idaho. If the results of this single season's study are representative of average conditions—and there is no evidence to the contrary—the following dates are important.

Near their upper altitudinal limits the radii of *Larix* become relatively stable after mid-July, *Pinus* after August first, *Thuja* after mid-September, and *Abies* after mid-October. At its lower limits the radii of *Abies* remain fairly constant after mid-August. *Thuja*, at the lower station, exhibited a second growing season, which probably lasted beyond mid-October. With this tree, as well as with *Larix* and *Pinus*, it appears that reasonably significant measurements of annual increment at low altitude cannot be made until after the rainy season is well under way.

Summary

Radial changes were followed in 32 trees divided equally among four species of conifers growing on Thatuna Ridge in northern Idaho. Four trees in each species (*A. grandis*, *L. occidentalis*, *P. ponderosa*, and *T. plicata*) were located at relatively low altitude, and the other four at relatively high altitude. The period of observation extended from April 19 or May 15 until October 17, 1942.

In spring the most apparent effect of the vertically stratified climates on tree growth consisted of a well-marked retardation of cambial activity at high altitudes, which was probably due to the temperature gradient. In summer and early autumn the water-balance gradient exerted the most evident influence, as shown by differences in the amount of shrinkage. At low altitudes the trunks invariably shrank in late summer. At high altitudes shrinkage was either less pronounced or not evident.

The higher the altitude, the later the trees attained their maximal growth rates, and the later the effects of dry summer weather became evident. Pronounced differences in behavior were observed with as little as a 227-meter difference in elevation. This was correlated with differences in location with respect to vegetation zones and suggests a possible practical application of a knowledge of zonation.

The time when radial enlargement is completed varies widely among the four species and is strongly affected by altitude. In general, stability is attained at a later date at low altitude because the hydration of tissues formed in summer is deferred until after the rainy season begins in autumn.

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SOME EFFECTS OF ALTITUDE AND WATER SUPPLY ON THE COMPOSITION OF DERRIS ELLIPTICA

RUFUS H. MOORE[1]

Introduction

The effects of altitude and water supply on *Derris elliptica* (Wall.) Benth. have received only brief mention in the literature on this tropical legume. BUNTING and MILSUM (2) reported that plants flourished at 4750 feet above sea level at 4°28′ N. Lat. in Malaya but did not comment on the quality of root produced at this elevation. Growing *Derris* at more than 2300 feet in the Netherlands East Indies was not recommended (4). Although 80 or more inches annually of uniformly distributed rainfall have been considered a requirement for this species, supporting data are notably lacking. Records from Puerto Rico and Guatemala, presented herewith, show the influence of altitude and, to a limited degree, of water supply on the composition of derris root.[2]

Sources of samples

Single-plot tests conducted in several regions of Puerto Rico from 1940 to 1942 showed the effects of different combinations of environment on the quality of derris root (10). Each plot contained from 225 to 275 transplants of the Sarawak Creeping variety, spaced 2 × 3 feet. A 12-8-5 commercial fertilizer was applied at the rate of 800 pounds per acre when these plants had resumed growth. The vines were allowed to trail naturally on the ground. The pH of the first and second foot of soil was determined with a Beckman pH meter when the vines had begun to form a soil cover. At harvest time all plants in a strip at least 6 feet wide were discarded to minimize border effects that had developed during the growth period. Only the roots of the 100 plants that had been inclosed by this border were analyzed. All samples in these regional trials and in the commercial plantings described in the next paragraph had some roots thicker than 1 cm.

In June, 1942, small commercial plantings of the Sarawak Creeping variety were made at three different elevations in Puerto Rico. In these fields exceptionally well-rooted plants were spaced 3 × 3 feet, and no fertilizer was used. Nineteen months later, samples were dug, each being a composite of part of the root systems of ten plants.

In August, 1940, the Department of Agriculture of Guatemala introduced from the Philippine Islands a variety of

[1] The author was formerly Plant Physiologist at the Federal Experiment Station, Mayagüez, Puerto Rico.

[2] All pH determinations and analyses for total chloroform extractives and rotenone were made by M. A. Jones, Chemist, Federal Experiment Station, Mayagüez, Puerto Rico. Mr. Jones and Gilda C. Vicente, Collaborating Agronomist, performed the carbohydrate analyses co-operatively.

Derris that closely resembles Sarawak Creeping. During the following spring, small lots of young plants were distributed to farmers on the Pacific watershed. Root samples were gathered in May, 1943, from plants supported on poles at elevations ranging from 910 to 4888 feet above sea level.[3] Portions of the root systems of from seven to ten plants were composited for each sample. Roots from higher altitudes were so much thinner than those from lower elevations that it seemed advisable to sort each sample into small and large roots. Small roots were less than 1 cm. in diameter, and large roots were 1 or more cm. thick.

TABLE 1

ANALYSIS OF DERRIS ROOT GROWN UNDER DIFFERENT ENVIRONMENTAL CONDITIONS IN PUERTO RICO

Period	Site	Feet above sea level	Inches of annual rainfall*	Soil			Months at harvest	Per cent of total chloroform extractives†	Per cent of pure rotenone†	Per cent of carbohydrates†
				Class	pH					
					First foot	Second foot				
1940 to 1942	Maricao	2400	98.9	Nipe clay	4.9	5.3	27	6.2	2.0	27.4
	Cidra	1400	79.1	Cialitos clay	4.7	4.7	24	13.3	4.3	19.0
	Utuado	380	73.4	Toa silty clay loam	6.1	5.4	28	19.6	6.0	.....
	Sabána Grande	220	49.1	Jácana clay	6.8	7.3	24	16.4	4.5	10.9
	Vega Baja	215	70.2	Bayamón fine sandy loam	4.7	5.4	28	20.7	6.0	5.9
	Mayagüez	50	76.3	Toa silty loam	6.9	7.2	27	21.3	6.8	13.1
1942 to 1944	Orocovis	1970	68.9	Mucara silty clay loam	.....		19	5.5	1.9	37.8
	Cidra	1410	89.7	Cialitos clay	acid		19	10.4	3.9	28.3
	Humacao	100	91.2	Humacao clay	acid		19	17.4	6.2	20.4

* The actual mean annual rainfall of the experimental period is given, except in the case of Cidra, for which incomplete 1940–1942 data made it necessary to use the mean rainfall over a period of years.

† Moisture-free basis.

Chemical methods

All roots were washed free of soil, and those thicker than 5 mm. were split lengthwise to facilitate drying. The samples became snap-dry within 3–5 days when spread out in a thin layer in indirect light. This material was ground in a no. 2 Wiley mill until fine enough to pass through a 0.5-mm. sieve.

The powdered samples, which contained from 5.8 to 11.8 per cent of moisture, were stored in tightly stoppered bottles until analyzed for total chloroform extractives (5), rotenone (1), and reserve carbohydrates. Sucrose was hydrolyzed with dilute HCl (1), and starch was digested with malt diastase followed by dilute HCl (1). The percentages of total sugars and of starch, estimated separately by the Lane-Eynon method (1), were added to give the values reported for reserve carbohydrates. "Hemicelluloses," determined on samples from the highest and lowest sites, varied from 6.1 to 9.3 per cent on a moisture-free basis. Since they showed

[3] The writer made these collections while on temporary assignment with the Office of Economic Warfare of the Foreign Economic Administration.

TABLE 2

ANALYSIS OF DERRIS ROOT HARVESTED IN 1943 AT SEVERAL ALTITUDES IN GUATEMALA

Site	Feet above sea level	Inches of annual rainfall*	Months at harvest	Per cent of total chloroform extractives†			Per cent of pure rotenone†			Per cent of reserve carbohydrates†		
				Small roots	Large roots	All roots	Small roots	Large roots	All roots	Small roots	Large roots	All roots
Guatemala City	4888	43.1	33	7.4	5.4	6.7	2.3	1.4	2.0	35.3	41.2	37.1
Finca Montevideo	3650	204.4	14	11.2	‡	11.2	3.5	‡	3.5	.....	.....	.....
Finca María Santísima	2050	99.0	24	17.7	11.7	16.1	7.0	4.4	6.3	19.8	36.2	24.2
Finca El Baúl	1700	178.8	24	16.6	11.3	14.6	6.3	3.9	5.3	21.9	32.8	26.5
Finca Velásquez	910	.....	25	17.2	12.5	14.7	6.0	5.0	5.5	28.4	37.9	33.2

* The actual mean annual rainfall for the experimental period is given, except an 11-year average for Finca El Baúl. Data for Finca Velásquez was incomplete.

† Moisture-free basis. ‡ No large roots in this collection.

no correlation with the known nutritional differences in the plants, complete analytical data on them have been omitted.

Results

Shoot development—luxuriant at lower sites with an adequate moisture supply—was observed to be progressively less vigorous as altitude increased or as water supply was restricted. In Puerto Rico, root nodules developed at all altitudes, but they were much larger at lower elevations. In Guatemala, no nodules were found on the roots collected at 4888 and 3650 feet, but they occurred in abundance on plants at elevations of 2050 feet or less.

Soil and climatological data and the chemical analyses of roots appear in tables 1, 2, and 3. Figures 1 and 2 correlate trends in the data.

Discussion

Both total chloroform extractives and rotenone are sometimes used to estimate the quality of derris root. Since variation in these two constituents followed similar trends, rotenone only will be used in this discussion as an index to root quality.

Altitude and temperature

Figure 1 shows that, in general, the quality of derris root was inversely correlated with altitude and that roots were

TABLE 3

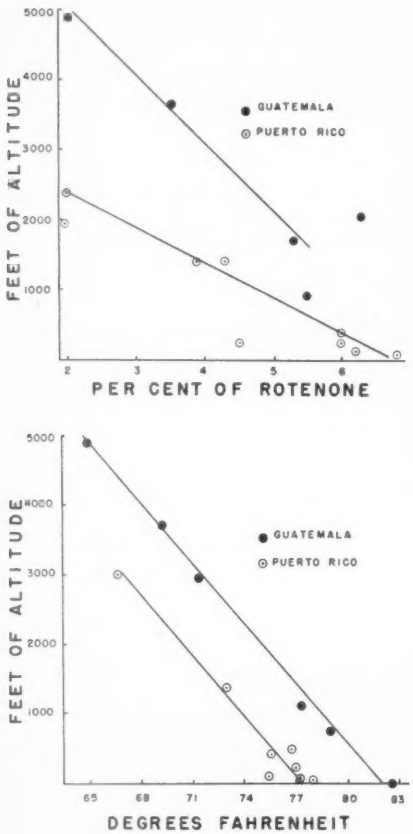
MEAN ANNUAL TEMPERATURES AT SEVERAL ELEVATIONS IN PUERTO RICO AND IN SOUTHWESTERN GUATEMALA

Country and station	Feet above sea level	Years	Mean annual temperature (°F.)
*Puerto Rico:**			
Guineo Reservoir	3000	15	66.7
Cayey	1400	41	73.0
Comerío Falls	500	37	76.8
Utuado	430	22	75.6
Manatí	250	42	77.0
Humacao	100	37	75.4
Mayagüez	80	43	77.2
San Juan	50	44	78.0
Guatemala:†			
Guatemala City	4888	13	64.9
Palín	3707	10	69.3
Mocá	2986	10	71.4
Escuintla	1115	10	77.4
Retalhuleu	787	10	79.0
Puerto San José	0	12	82.8

* Data from U.S. Department of Commerce, Weather Bureau's *Climatological Data: West Indies and Caribbean Section*, 22, No. 13, 105. 1943.

† Data, secured through the co-operation of the Foreign Economic Administration's office in Guatemala City, was furnished by the courtesy of the National Observatory in Guatemala.

consistently higher in rotenone at greater altitudes in Guatemala than in Puerto Rico. Obviously, one or more factors associated with altitude caused these gradients of response in the derris plants.



FIGS. 1–2.—Fig. 1, relationship between altitude and percentage of rotenone in derris root. Fig. 2, relationship between altitude and mean annual temperature.

Any correlation of rotenone content with intensity, duration, or quality of sunlight or with relative humidity cannot be considered because adequate meteorological records are not available. Inasmuch as mean temperature decreases as altitude increases, temperature could be a critical factor in the altitude effect. This possibility is indicated in figure 2, which shows that zones of the same mean annual temperature averaged from 1200 to 1300 feet higher in Guatemala than in Puerto Rico.

Geography accounts for higher temperatures at the same elevations in Guatemala than in Puerto Rico. The part of Guatemala from which roots were collected lies at approximately 14°40′ N. Lat., 3°35′, or about 247 miles nearer the equator than does Puerto Rico. Also, the Pacific watershed of Guatemala is protected from northerly winds by volcanic ranges and is part of a relatively large land mass on which temperatures are not moderated to the extent that they are in Puerto Rico, a small land mass in the direct path of the trade winds (**7, 13**). The inherently tropical adaptation of *Derris* shows clearly in its response to the temperature relation of altitude.

WATER SUPPLY

Although there was a close inverse correlation of root quality with elevation and temperature in the upper range of altitudes, divergent cases were found at lower elevations. Certain differences in water supply were associated with and probably caused these exceptions.

Rain was the only source of moisture for the derris plantings in Puerto Rico. Precipitation at the test sites can be roughly classified into two rainfall-distribution types. One type has a 4–5-month period of definite drought, and the other has appreciable rainfall in the so-called dry season. Maricao, Sábana Grande, and Mayagüez were characterized by seasons of definite and protracted drought. Orocovis, Cidra, Vega Baja, and Humacao received enough

rain to promote growth during the drier part of the year. Although Utuado did not fit readily into either type of rainfall distribution, its dry season was broken by fairly heavy rains. Vega Baja was outstanding in the uniformity of rainfall distribution, there having been only 3 months of the 28 in the crop cycle that had less than 2.6 inches. The rainfall-distribution patterns just described were too similar to exert an unmistakable effect on root quality.

Differences in total annual precipitation appear to have had no measurable effect on root quality at higher altitudes in Puerto Rico (see table 1), for roots of almost identical rotenone content were grown with 98.9 and 68.9 inches of rainfall at Maricao and Orocovis, respectively. But total annual precipitation was effective at lower elevations. For example, roots from Sábana Grande, where the mean annual rainfall was only 49.1 inches, were much lower in rotenone than those grown at similar low elevations receiving 70.2 and 73.4 inches of yearly precipitation.

Unlike Puerto Rico, the Pacific versant of Guatemala has only one rainfall-distribution pattern. The 6-month rainy season beginning in May is followed by 6 months of little or no rain (7). But, as one proceeds from the highlands toward the lowlands, mean annual precipitation decreases, and the dry season becomes intensified. It is during this part of the year that some fields are irrigated from the numerous mountain streams that transect the watershed.

No irrigation was used at altitudes of 4888, 3650, and 1700 feet. The derris root from these elevations showed a definite inverse correlation between rotenone and altitude. The extent to which irrigation supplemented rainfall had considerable bearing on the quality of root secured from two of the sites within the range of altitudes ecologically favorable to *Derris*. Irrigated twice monthly during both dry seasons, the plants at 2050 feet had an almost uniform distribution of water throughout the year. As a result, they grew luxuriantly and produced the highest-quality root harvested in Guatemala. By contrast, the plants at 910 feet were irrigated only four times in the first dry season and three times in the second. Insufficient irrigation, especially in the second dry season, was probably the cause of the relatively low percentage of rotenone at this elevation.

Other factors

Although most of the soils in Puerto Rico on which *Derris* was grown are classified as clay or clay loam, they were of good physical condition because of a definite crumb structure. Differences in the pH of these soils had no apparent effect on the quality of derris root (see table 1). Volcanic in origin, the Guatemalan soils are rich, deep, and well drained. Some of those examined at higher altitudes have a considerable admixture of fine cinders, but those at lower elevations have the texture of silty loam or fine sandy loam. On the whole, there was more variation among the Puerto Rican soils than among the Guatemalan soils. The data presented are insufficient to indicate whether soil type in either country appreciably affected the quality of derris root.

Although the plants grown in both countries had the characteristics of the Sarawak Creeping variety, they were sufficiently different from each other to be two distinct intravarietal selections. Apparently, they responded differently to the decline in temperature with altitude. Figure 2 shows that the temperature gradients in both countries were ap-

proximately equal to the normal lapse rate of 1° F. fall in temperature with each 300 feet of vertical ascent (3). However, figure 1 shows that, as elevation increased, the quality of derris root was not lowered so rapidly in Guatemala as in Puerto Rico. This might indicate that the derris selection in Guatemala is better adapted to ecologically higher altitudes than the Sarawak Creeping selection of Puerto Rico.

It is possible that the methods of vine management employed in the two countries lessened the observed differences in the quality of root produced at the same elevation. Guatemalan *Derris* was trained onto poles, while Puerto Rican *Derris* was allowed to trail over the soil. As the use of vine supports lowers the percentage of rotenone in the roots (9), the use of poles in Guatemala probably did not favor the production of roots of maximum quality.

Harvest age affects the quality of derris root grown at a favorable altitude. The extent to which the rotenone content can be expected to change with age was demonstrated in the Sarawak Creeping variety at Mayagüez, where the percentage of rotenone rose from 5.0 at 18 months to 5.7 at 24 months and then declined to 5.2 at 27 months (10). Roots produced at low and medium elevations in Puerto Rico exemplified part of this normal change in analytical values. Roots harvested near Cidra when 19 and 24 months old had 3.9 and 4.3 per cent of rotenone, respectively. Similarly, 19-month-old roots from Humacao had 6.2 per cent of rotenone in comparison to 6.8 per cent in 27-month-old roots from Mayagüez. On the other hand, the few data that are available indicate that the age effect was practically obscured at higher altitudes. In Puerto Rico, the difference between 1.9 and 2.0 per cent of rotenone in roots 19 and 27 months old, respectively, falls within the limits of analytical error. In Guatemala the minimum difference of 43 per cent between 3.5 and 2.0 per cent of rotenone in roots 14 and 33 months old is much greater than the normal variation in root quality in this range of ages.

Rotenone-carbohydrate relationships

A pronounced restriction of the water supply (6) or a lowering of temperature (12), either of which increases the carbohydrate reserves in other species of plants, causes a similar response in *Derris* (tables 1, 2, and 3). Even though carbohydrates are not the basis for evaluation of derris root as an insecticide, they are of special interest because they normally have a reciprocal relationship to rotenone.

Rapid growth of derris plants causes increased elaboration of rotenone and less storage of carbohydrate reserves, but slow growth is accompanied by relatively slow deposition of rotenone and comparatively rapid accumulation of carbohydrates (11). Rotenone and carbohydrate reserves maintain this inverse relationship when the nutritional level of the plant does not fluctuate excessively. This normal effect of growth vigor on rotenone-carbohydrate relationships is clearly evident in the Puerto Rican harvests of 1944. It was observed that shoot growth of the plantings at Orocovis, Cidra, and Humacao was of practically the same order as the rotenone percentages in the roots, but the carbohydrate percentages were in the reverse order. The same rotenone-carbohydrate relationship also appears in the 1942 Puerto Rican data for Maricao, Cidra, and Mayagüez and in the 1943 data for Guatemala City and Finca El Baúl.

Because reserve carbohydrates, but not rotenone, can be utilized in normal metabolic processes of the derris plant (8), the rotenone-carbohydrate relationships may not always be those just described. In general, the extent of carbohydrate accumulation in Puerto Rican plants varied with the season. The 1944 harvests were made after the dry season had begun, when growth had been checked and carbohydrates had accumulated to relatively high percentages. On the other hand, the 1942 harvests were made during the rainy season or shortly after the onset of the dry season, when rapid growth was utilizing carbohydrate reserves. Sudden increases in moisture several days prior to the Vega Baja and Sábana Grande harvests initiated growth flushes that reduced carbohydrate reserves to exceptionally low values.

Inasmuch as the root samples from Guatemala were gathered at the end of the dry season, all were high in carbohydrates. Nevertheless, the effect of water supply was reflected in the concentration of these reserves. The frequent irrigation of plants grown at 2050 feet produced vigorous growth that prevented carbohydrates from accumulating to the extent that they would have if rainfall had been the only source of water at this elevation. By contrast, the less frequent application of water to plants at 910 feet, in conjunction with the more intense dry season at this lower elevation, allowed carbohydrates to increase to a value higher than would be indicated by their rotenone content. Tip-burned leaves were evidence that these plants had suffered from serious water shortage during the last few months prior to harvest.

Summary

1. *Derris elliptica* was grown in Puerto Rico at elevations ranging from 50 to 2400 feet above sea level and in Guatemala at altitudes ranging from 910 to 4888 feet.

2. The temperature factor of altitude correlated positively with rotenone and inversely with reserve carbohydrates in derris roots.

3. At elevations favorable to growth of derris plants, the accumulation of rotenone was influenced by major variations in water supply, but reserve carbohydrates were altered by relatively minor variations in available moisture.

4. The varietal selection of *Derris* grown in Guatemala appeared to be more adapted to higher elevations, in an ecological sense, than the Sarawak Creeping variety grown in Puerto Rico.

5. The normal effect of age on the percentage of rotenone was apparently obscured at high altitudes.

6. Soil pH, ranging from values of 4.7 to approximately neutral, had no measurable effect on rotenone or carbohydrate reserves.

7. At favorable altitudes, derris plants flourished in soils having good physical structure and belonging to several distinct soil types.

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STUDIES ON PLANT GROWTH-REGULATING SUBSTANCES

This issue of the BOTANICAL GAZETTE contains eighteen papers originating at Camp Detrick, Frederick, Maryland. The work reported therein was carried out as a part of the activities of the Special Projects Division, Chemical Warfare Service, in 1944 and 1945. Earlier publication of this material was incompatible with wartime security policies determined by the War Department.

This work was developed from the base laid by many workers on growth-regulatory and formative effects produced by hetero-auxin, naphthalene acetic acid, and similar or related compounds. As a result of various experiments in the late thirties and early forties, the high activity of certain of these synthetic growth-regulating substances was recognized. This led to the suggestion by E. J. Kraus that they might find use for herbicidal purposes and thence to the initiation of tests in which inhibitory potency was established.

Other laboratories and groups were also involved in the project on a contract basis. J. W. Mitchell, of the Bureau of Plant Industry, United States Department of Agriculture, Beltsville, Maryland, participated from its initiation and, together with E. J. Kraus, carried out in February, 1944, at the University of Chicago, certain key experiments that provided unequivocal proof of the herbicidal activity of a few substituted phenoxyacetic and benzoic acids. Melvin S. Newman, Department of Chemistry, Ohio State University, was responsible for the synthesis of a substantial number of compounds for test. All these individuals conformed to the security requirements of the project and consequently withheld from publication their findings. In some instances, also, publication of the results of other related physiological work upon which they were currently engaged was delayed. The subject of the synthetic plant growth-regulating substances and, in particular, their application for herbicidal purposes will therefore not be represented in the scientific literature in wholly correct chronology. In this connection it must be borne in mind also that important developments in this field took place entirely independently in Great Britain.—A. G. NORMAN.

CAMP DETRICK
FREDERICK, MARYLAND

NEW GROWTH-REGULATING COMPOUNDS. I. SUMMARY OF GROWTH-INHIBITORY ACTIVITIES OF SOME ORGANIC COMPOUNDS AS DETERMINED BY THREE TESTS[1]

H. E. THOMPSON, LT. (J.G.), U.S.N.R.; CARL P. SWANSON, LT., U.S.N.R.; AND A. G. NORMAN

Introduction

The systematic study of organic compounds of known structure in relation to physiological effects on plants may be said to have been initiated in 1931, when F. KÖGL and his collaborators—HAAGEN SMIT, ERXLEBEN, TONNIS, and KOSTERMANS—began investigations on the identification of the *Wuchsstoff* (**29**). In 1935, KÖGL (**28**) published the assigned structure of auxentriolic acid (auxin-a) and auxenolonic acid (auxin-b) and noted the similarity between the physiological activities of hetero-auxin (beta-indoleacetic acid) and the auxins. This observation was confirmed by THIMANN and KOEPFLI (**43**).

KÖGL (**28**) and KÖGL and KOSTERMANS (**30**) used the *Avena* coleoptile test for the determination of regulating activity of some thirty-four substances, including derivatives of the auxins, hetero-auxin, 2- and 3-indolecarboxylic acid, beta-(3-indole)-propionic acid, 3-indole-pyruvic acid, and beta-(3-indole)-alpha-amino-propionic acid. HAAGEN SMIT and WENT (**14**) investigated three different methods for testing physiological activity and studied thirty-three substances, mostly indole derivatives, substituted phenylacetic and cinnamic acids, and some alkyl carboxylic acids. THIMANN (**41**) presented an analysis of the activity of 3-indoleacetic acid, and the analogous indene and coumaryl (benzofuran) derivatives on plant tissues. Various indole-substituted aliphatic acids were tested by BAUGUESS (**1**) for root initiation, stem bending, and bud inhibition of tomatoes, marigolds, and stocks. HITCHCOCK (**19, 20**) reported the effects of indoleacetic, phenylacetic, and naphthaleneacetic acids and several of their homologues on leaves of the tobacco plant. ZIMMERMAN and WILCOXON (**59**) tested alpha- and beta-naphthaleneacetic acid, 5-acenaphtheneacetic acid, gamma-indolebutyric acid, phenylacetic acid, 3-indoleacetic acid, fluoreneacetic acid, anthraceneacetic acid, alpha-naphthaleneacetonitrile, gamma-(2-carboxy-3-indole)-butyric acid, and indolepropionic acid on sweet peas and other plants.

The root-initiating activity of a variety of indoleacetic and similar compounds was tested by ZIMMERMAN and HITCH-

[1] These studies were carried out between February, 1944, and September, 1945, as part of a project at Camp Detrick, Md., under the general direction of Dr. A. G. Norman and with the advice and assistance of Dr. E. J. Kraus, University of Chicago.

Many persons participated in this work in various degrees and capacities, but especially to be mentioned are the following: J. F. Owings, Jr., Ph.M. 1/C, U.S.N.R.; John H. Wotiz, P.f.c., and Eric J. Smith, T/5, who, under the direction of H. E. Thompson, Lt. (j.g.), U.S.N.R., prepared at Camp Detrick the bulk of the compounds listed; William S. Fones, John H. Wotiz, and Mary Renoll, of the Department of Chemistry, Ohio State University, who, under the direction of Dr. Melvin S. Newman, prepared those compounds marked (*) in the tables; Dr. John W. Mitchell, of the Bureau of Plant Industry, U.S. Department of Agriculture, who aided in the establishment of the program, and, together with Dr. J. W. Brown, conducted Test B at Beltsville, Md.; Daniel Ready, Capt., A.U.S., who, with the assistance of G. B. Landry, Cpl., conducted Test A at Camp Detrick and maintained the test records; Joseph Scherr, T/5, who, under the supervision of C. P. Swanson, Lt., U.S.N.R., carried out Test C at Camp Detrick.

COCK (47). This was extended by HITCHCOCK and ZIMMERMAN (21) to include studies of the absorption and movement of such synthetic growth substances from soil, as judged by the responses produced in the aerial parts of the plants, and of the rooting responses of cuttings (22). Skatole (3-methylindole) was tested as a growth-regulating substance by GLOVER (12). Alkyl esters of alpha-naphthaleneacetic, phenylacetic, indoleacetic, indolepropionic, and indolebutyric acids were compared in activity on tomato, sunflower, and other plants by ZIMMERMAN, HITCHCOCK, and WILCOXON (57).

GAVAUDAN, GAVAUDAN, and POMRIASKINSKY-KOBOZIEFF (11) in 1937 published observations on the effects of colchicine on onion roots, and HAVAS (17, 18) the responses of wheat seedlings to the same compound. In the same year ZIMMERMAN (46) reviewed the responses of plants to hormone-like substances and, with HITCHCOCK (48), tested the relative effectiveness of inorganic and organic salts of alpha-naphthaleneacetic, beta-indoleacetic, beta-indolepropionic, and gamma-indolebutyric acid on tomato and other plants, bending responses, root initiation, retardation of growth, and swelling of treated tissues all being used in the evaluation. THIMANN (42) studied the nature of the inhibitions caused by the application of auxin to *Pisum* seedlings. GARDNER and MARTH (10) described the development of parthenocarpic fruits following spray treatment with various growth-promoting substances in very low concentrations.

The histological reactions produced in bean plants by some growth-regulating compounds were investigated by HAMNER and KRAUS (15). BEAL (3) described the bud development in *Lilium harrisii* caused by treatment with indoleacetic acid and later extended this to a study of the histological responses produced in three species of *Lilium* by treatment with the same substance.

ZIMMERMAN and HITCHCOCK (49) investigated the tropic responses of leafy plants treated with growth-regulating substances and later (50) the combined effects of light and gravity on the responses of plants so treated. HITCHCOCK and ZIMMERMAN (23) described the use of tomato and other cuttings in the determination and evaluation of physiological activity. ZIMMERMAN, HITCHCOCK, and WILCOXON (58) gave the results of tests of twenty-nine substances, including alpha- and beta-naphthoxyacetic acids applied as vapors or in solution to corn, tomato, pea, and other plants. Nastic curvatures, tropic responses, swellings, root initiation, retardation or inhibition, and parthenocarpy were all described. BAUSOR (2) reported the effects of beta-naphthoxyacetic acid on tomato plants, and MULLISON (34) the responses of bean plants to tetrahydrofurfural butyrate. BLOCH (8) discussed the use of synthetic growth-regulating substances on plants.

The number of naphthoxyacetic acid derivatives demonstrated to have plant growth-regulatory activity and to produce formative growth effects was considerably extended by ZIMMERMAN (44) and by ZIMMERMAN and HITCHCOCK (51, 52). In 1942 the same authors (53, 54) reported the testing of halogen, nitro-, and amino-substituted phenoxyacetic and benzoic acids on a variety of plants with particular reference to epinasty, modification of organs, initiation of adventitious roots, inhibition of buds, formative influences, and parthenocarpic fruit setting. By this time a sufficient number of substituted phenoxy compounds had been studied to permit in-

vestigation of the relationships between chemical structure and root-inducing activity (**24, 25, 26**). ZIMMERMAN (**45**) further reviewed the information available on the formative influence of growth-regulating substances on plants. MARTH (**32**) described experiments on the effects of a number of naphthalene and indole derivatives on shoot development of roses.

In 1944 various workers (**5, 16, 33**) independently suggested in print the use of synthetic growth-regulating substances as selective herbicides. Not in every case, however, was proof of herbicidal or phytotoxic action given, nor was selective activity demonstrated. MITCHELL and HAMNER (**33**) described the use of certain polyethylene glycols as carriers in aiding in the preparation of aqueous solutions of some of the more insoluble growth-regulating substances. The results of applications of thirty-one substances of this type in the aerosol form were reported by ZIMMERMAN and HITCHCOCK (**55**); and later the same workers, with HARVILL (**56**), tested fourteen substituted xylenoxy acids for activity comparable to the phenoxy acids.

SLADE, TEMPLEMAN, and SEXTON (**37**) in 1945 published a list of thirty-two aryloxyacetic acid derivatives that had been tested for herbicidal activity, some, it was stated, as early as 1941. They drew attention particularly to the high promise of 2-methyl-4-chlorophenoxyacetic acid for this purpose. BLACKMAN (**7**) compared the behavior of this acid with that of 2,4-dichlorophenoxyacetic acid as a selective herbicide, and NUTMAN, THORNTON, and QUASTEL (**35**) described experiments on these two substances in soil.

In the same year SYNERHOLM and ZIMMERMAN (**38**) prepared 2-chloro-3,5-diiodo- and 2-chloro-3,5-dibromobenzoic acids and investigated the effects produced by them on tomato plants and also (**39**) reported tests of forty-six aryloxy-alkyl carboxylic acids for plant growth-regulating activity, using as criteria the threshold concentrations in lanolin causing cell elongation and formative effects. BEAL (**6**) continued histological studies of tissues of plants treated with certain phenoxyacetic compounds.

Plant growth-regulating activity of a somewhat different character has been found to be a property of certain compounds of the urethane series. The earliest tests reported in the literature appear to be those of FRIESEN (**9**) in 1929, who investigated the action of phenyl and ethyl urethane on oat and wheat seedlings. LEFÈVRE (**31**) commented on the similarity of the reactions caused in some plants by colchicine and phenyl urethane. SIMONET and GUINOCHET (**36**) and GUINOCHET (**13**) also noted that phenyl urethane and certain other compounds caused colchicine-like changes in wheat and wheat seedling. A number of urethanes were described by TEMPLEMAN and SEXTON (**40**) in 1945 as possessing some activity in causing inhibition of cereal seedlings. Isopropylphenylcarbamate was said to be the most active of these relatively insoluble compounds.

Early in 1944 a project centered at Camp Detrick, Maryland, was initated for the study of inhibitory activities of synthetic plant growth-regulating substances. By August, 1945, almost eleven hundred substances had been synthesized and submitted to test in the laboratory and greenhouse. The more promising substances were further studied in greenhouse and field on a wide range of plant species. In this paper are summarized the experimental data obtained in screening these compounds by three tests employed routinely. All compounds are

included, whether proved to have activity or not.

Methods

Considerable diversity exists between methods used by investigators for the evaluation of compounds as plant growth-regulating substances (**9, 14, 19, 20, 21, 22, 23, 27, 33, 39, 41, 48, 55, 58**), but none of these was satisfactorily applicable to the objectives of this project. In most cases the results of previous tests were not capable of quantitative expression in such a manner as to permit comparison of activities of groups of substances tested at different times. Perhaps most closely approaching the requirements was the device of establishment of threshold concentrations of any compound necessary to cause the appearance of some readily recognizable plant reaction. This has been principally used by HITCHCOCK and ZIMMERMAN in their work. Since, however, gross inhibition of growth was considered to be the most likely manifestation of phytocidal activity in this class of compound, the effects of which are telemorphic and in great contrast to those produced by contact herbicides, all tests were based on weight or length changes following treatment with a uniform quantity of the compound under test. In order that seasonal or environmental effects should be minimized, a common reference material was employed in all test groups, and the results of any test were expressed as a percentage of the inhibition produced concurrently by the reference material. The reference material used in each of the three tests reported in this communication was 2,4-dichlorophenoxyacetic acid, commercial grade purified through the ammonium or alkali metal salt by several recrystallizations from aqueous and alcoholic solutions.

The primary test involved the determination of inhibition of elongation of the primary root of germinating corn. This method in detail follows.

TEST A. CORN-GERMINATION TEST

Corn grains of the variety of Silver King (Wisconsin No. 7) were germinated at a constant temperature of 27° C. on filter paper in covered 6-inch Petri dishes, to which were added 20 ml. of aqueous solution[2] of the compound to be tested, at a concentration of 10 p.p.m. Twenty-five seeds were used in each dish, and three dishes were used with each compound. After 4 days of growth, the length of the primary root was measured. Inhibition of growth was determined by subtracting the average length of the primary root of the treated seeds from that of the control or untreated seeds. Inhibition of growth caused by the application of 2,4-dichlorophenoxyacetic acid was arbitrarily designated as 100%, and inhibition of root growth resulting from all other compounds was compared in terms of this percentage. Treatments with 2,4-dichlorophenoxyacetic acid were included in each group of tests.

In addition, two other methods were employed for the evaluation of the plant growth-regulating activity of chemical compounds and are reported for purposes of additional information. The methods were based on the application of a single drop of solution of the compound under test to young kidney-bean plants, under greenhouse conditions, and measurement of the effect of the treatment upon the subsequent vegetative growth. In one test the compound was applied in aqueous solution and in the other in oil, a

[2] In some cases it was found necessary to add small amounts of extremely dilute solutions of ammonium hydroxide or of acid to effect complete solubility of the compound for this test.

co-solvent being used where necessary. The details of these tests follow.

TEST B. THE KIDNEY-BEAN SINGLE-DROPLET WATER TEST

Kidney-bean seeds were planted in 4-inch pots containing approximately 1 pound of composted soil. Seven to 10 days after planting, the seedlings were approximately 4 inches in height and had developed primary leaves that were about $1\frac{1}{2}$ inches in width. The pots were then thinned to one plant per pot, nine plants being treated with each test substance. Each plant was treated with 0.02 ml. of an aqueous solution containing 200 p.p.m. (4 γ) of the compound. This solution included sufficient Carbowax No. 1500 to furnish a 0.5% solution of the wax. The treatment was applied to the upper surface of one of the primary leaves at a point along the midrib and approximately $\frac{1}{8}$ inch from the point of attachment of the blade and petiole, a $\frac{1}{10}$-ml. pipette graduated in hundredths being used to apply the droplet. The fresh weight of that portion of each plant above the second node was obtained the 10th day after treatment. Inhibition of growth due to the use of 2,4-dichlorophenoxyacetic acid was designated as 100%, and the effect of other compounds was compared on this basis. Untreated controls were included in each series of tests.

TEST C. THE KIDNEY-BEAN SINGLE-DROPLET OIL TEST

Essentially the same procedure was employed in the kidney-bean single-droplet oil test as in the kidney-bean single-droplet water test. The differences were (1) there were two kidney-bean plants per 4-inch pot (three pots per series); (2) the treatment was made at the time the primary leaves were fully expanded and the second internode was approximately 1 inch long (at which time the plant was approximately 2 weeks old); and (3) 0.01 ml. of solution was used in the treatment. The concentration of the solution was such that a drop (0.01 ml.) contained 5 γ in oil of the compound to be tested. When tributylphosphate was used as a co-solvent, as was the case with all compounds that were not directly oil-soluble or oil-miscible, the concentration of this substance was usually 0.2%. Harvest of fresh weights was effected in 10 days, and all calculations were carried out as in the kidney-bean single-droplet water test.

Discussion and Interpretation of Tests

In considering the results of the tests listed, consideration must be given to the reasons for establishment of each of the tests and the limitations inherent in them. Close numerical agreement between the results of the three tests is not necessarily to be expected, not only because different levels of growth activity may be manifest under the test conditions prevailing but also because in some cases the physical properties of certain of the compounds were such as to make difficult or impossible their testing at the desired concentration. For example, many compounds synthesized, though not completely water-insoluble, had such a low solubility that it was not found feasible to prepare with confidence for Test A a solution containing 10 p.p.m., which necessarily has to be done by diluting from a more concentrated solution. Many of these relatively insoluble compounds, however, could be handled in Carbowax to give the necessary 200-p.p.m. solution for Test B. Compounds of the classes of acid halides and acid anhydrides included in these tests may react with water to give the correspond-

ing organic acid. In such cases and in those instances when ammonium hydroxide or hydrochloric acid was used to effect solution in water, the possibility of some chemical change occurring must be taken into consideration.

All tests were carried out with adequate replication, and the results reported are means of the treatments. It was possible to examine statistically the level of significance within any one group of compounds tested concurrently. This, however, has not been done for the whole group, because the position of any one compound with respect to another is not absolute but is dependent to some extent on environmental and seasonal conditions. The degree of inhibition produced by the reference compound in Tests B and C at different times of year was not wholly identical and was affected by such factors as rate of growth of the test material. Test A was undoubtedly the most reproducible, and for that reason and because of its simplicity it formed the primary basis for detection of inhibitory activity. The errors introduced by variation in the replicates were, on the whole, much smaller when the activities of the compound under test were high. Minimum differences necessary for significance are therefore generally greater within Groups IV-A or IV-C than in Group I.

It is to be noted that all tests contain a quantitative factor and that therefore the absence of inhibitory activity in the test is not to be taken as proof of absence of growth-regulating properties. Many of the compounds in Group IV-C, though manifesting little or no activity at the levels tested, might well prove active at higher concentrations. Compounds of those groups possessing significant activity by one or more tests described may be used as herbicides. Others may have possessed growth-regulatory effects not manifest primarily in gross inhibition. None of these tests was designed to reveal formative effects quantitatively, though abnormalities in the growth habit of the plants were frequently observed in Tests B and C. Any consideration of relationships between structure and physiological activity should be conducted with these facts in mind.

In Test A it was sometimes found that the mean length of the roots of treated seeds was greater than that of the untreated control. Such results are recorded as a negative value for inhibition, expressed, as in the case of positive values, as a percentage of the inhibition effected by the reference material. Small negative values are probably not significant and are due to the normal variability of biological material. Larger figures, however, do indicate over-all growth stimulation, indirectly expressed by the system followed here. It would be desirable that such compounds be investigated further by more appropriate methods designed specifically to test for cell or tissue elongation or growth stimulation.

In Test B the value "0" was recorded in all cases in which the mean fresh weight of the new growth was equal to, or greater than, that of the untreated controls. Negative values, however, were recorded in Test C when greater growth was made by treated plants than by controls. Values greater than 100 occurred in a number of instances. By this it must be understood that the mean fresh weight of the new growth of treated plants exceeded that of the controls by an amount more than equal to the inhibition produced by the reference compound. In such cases the test compound did not necessarily produce enhanced normal growth; frequently, there was tissue proliferation and gall production

at the growing-point. In general, inhibitory effectiveness on kidney-bean plants was accompanied by tissue deformation and abnormal proliferation at lower rates of application.

When the results of the tests are viewed as a whole, the corn-germination test (Test A) proved to be reliable in separating those compounds that possess high inhibitory activity for most broadleaved plants from those with little or no activity at the same concentration. It is paradoxical that activity on established monocotyledonous plants, and on the Gramineae in particular, cannot be predicted from this test. The aqueous single-droplet test on the kidney bean (Test B) proved also to be a reliable index of probable inhibiting or herbicidal activity of compounds applied in the form of aqueous sprays to many broadleaved species. In general, the results of this test agreed fairly satisfactorily with those of Test A, though the spread was somewhat greater and some discrepancies were found. These were almost all in the direction of a lower activity in Test B than in Test A. Examination of Group IV-A reveals very few compounds substantially more active by Test B than by Test A.

The oil single-droplet test (Test C) was established in order to evaluate oil-miscible compounds having little or no aqueous solubility and can be used as a basis for selection of inhibitory compounds for incorporating in oil sprays for herbicidal purposes. By the expedient of employing tributylphosphate as a co-solvent, it was found possible to include in this test many compounds not directly oil-soluble or oil-miscible. The reference material—2,4-dichlorophenoxyacetic acid—could itself be introduced into oil only in this way. Variation between replicates in this test was always greater than in Test A or Test B. However, the results obtained were satisfactory in separating active inhibitors from those of low or little activity. Subsequent spray tests usually confirmed predictions as to herbicidal activity on broadleaved plants made from the results of Test C. Many of the numerical values obtained in this test are substantially higher than those recorded for Tests A and B. This is explained by the fact that the reference material—2,4-dichlorophenoxyacetic acid, which was a fortunate choice for Tests A and B—stands somewhat lower in activity in oil with respect to many of the other compounds tested than it does in aqueous solution, even though its absolute activity when distributed in oil is certainly no lower than when in water. Some compounds, though fully water-soluble, were considerably more active in oil than in water.

Results

The results obtained in testing this series of compounds are given in Tables 1–6. The compounds are classified into groups according to the percentage of activity of 2,4-dichlorophenoxyacetic acid by the corn-germination test (Test A): Group I, 80% or higher; Group II, 50–79%, inclusive; Group III, 30–49%, inclusive; Group IV-A, 29% or lower by Test A, but 50% or higher by Tests B and/or C; Group IV-B, not sufficiently soluble for Test A, but 50% or higher by Test B and/or C; Group IV-C, other compounds tested. The compounds are listed alphabetically in each group.

Summary

1. The literature relating to the testing of organic compounds for growth-regulating activity on plants has been reviewed chronologically.

2. Three methods have been devised, whereby inhibitory activity at high dilu-

tion can be determined and evaluated. All depend on elongation or weight changes subsequent to treatment. The activity of the compounds under test are expressed as a percentage of the inhibition brought about concurrently by 2,4-dichlorophenoxyacetic acid as a common reference material. The plant materials used are germinating corn and young kidney-bean plants.

3. One thousand and sixty organic compounds, almost all synthesized expressly for this work, were submitted to test by the three methods. The data obtained have been assembled in groups on the basis of activity in reducing elongation of the primary root of germinating corn as compared with the reference material.

4. The significance and interpretation of the tests are discussed. Compounds showing high activity are indicated as promising for use as herbicides.

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TABLE 1

ACTIVITIES OF COMPOUNDS: GROUP I

Compounds possessing 80% or more of the activity of 2,4-dichlorophenoxyacetic acid in the corn-germination test (Test A), listed in alphabetical order. The results of the kidney-bean single-droplet water test (Test B) and the kidney-bean single-droplet oil test (Test C) are similarly expressed as the percentage of the activity of 2,4-dichlorophenoxyacetic acid in the same tests.

COMPOUND	TEST		
	A	B	C
2-bromo-4-chlorophenoxyacetic acid*	101	69	13†
butyl 2,4,5-trichlorophenoxyacetate	84	149	128†
2-chloro-4-bromophenoxyacetic acid*	80	84	52†
4-chlorocinnamic acid, ammonium salt*	90	23‡	.....
4-chlorophenoxyacetamide	84	174	ns§
3-chlorophenoxyacetic acid*	85	0	−158†
4-chlorophenoxyacetic acid*	85	107	162†
2,4-dichlorophenoxyacetamide	94	57	ns§
2-(2′,4′-dichlorophenoxyacetamido)-1-butanol	81	16‡	ns§
4-(2′,4′-dichlorophenoxyacetamido)-2,5-dichlorobenzenesulphonic acid, sodium salt	100	48	69†
2-(2′,4′-dichlorophenoxyacetamido)-2-ethyl-1,3-propanediol	88‖	96‡	12†
2-(2′,4′-dichlorophenoxyacetamido)-2-(hydroxymethyl)-1,3-propanediol	93	55	ns§
2-(2′,4′-dichlorophenoxyacetamido)-2-methyl-1,3-propanediol	8	102	12†
2-(2′,4′-dichlorophenoxyacetamido)-naphthalene-1-sulphonic acid	91	36	128†
1-(2′,4′-dichlorophenoxyacetamido)-naphthalene-8-sulphonic acid	92‖	29	ns§
1-(2′,4′-dichlorophenoxyacetamido)-8-naphthol-3,6-disulphonic acid	95	65	ns§
3,4-dichlorophenoxyacetic acid*	89	55	107†
2,5-dichlorophenoxyacetic acid*	96	8	−52†
2,4-dichlorophenoxyacetic anhydride	102	13	97†
2,4-dichlorophenoxyacetic 4′-sulphoanilide	104	0	30
2,4-dichlorophenoxyacetohydroxamic acid	95	65	ns§
2,4-dichlorophenoxyacetyl chloride	107	58‡	137†
2,4-dichlorophenoxyacetyl guanidine	101	89	ns§
N-(2,4-dichlorophenoxyacetyl) urea	99	117	ns§
alpha-(2,4-dichlorophenoxy)-butyric acid*	96	7	92†
beta-(diethylamino)-ethyl 2,4-dichlorophenoxyacetate*	100	84	90†
beta-(diethylamino)-ethyl 2,4,5-trichlorophenoxyacetate*	104	53	200†
2,2-dimethyl-1,3-dioxalane-4-methanyl-2′-methyl-4′-chlorophenoxyacetate*	100	3‡	−22
1,4-di-(2′,4′,5′-trichlorophenoxyacetamido)-benzene	100	102	ns§
,3-di-(2′,4′,5′-trichlorophenoxyacetamido)-benzene	99	89	ns§
ethyl 2,4-dichlorophenoxyacetate*	96	100	66
ethyl 2-methyl-4-chlorophenoxyacetate*	99‖	‡	118
ethyl alpha-(2-methyl-4-chlorophenoxy)-heptoate*	85‖	0‡	−50
beta-hydroxyethyl 2,4-dichlorophenoxyacetate*	94¶	117	93
2 iodo-4-chlorophenoxyacetic acid*	88¶	0	14†
2-methyl-4-bromophenoxyacetic acid*	92	0	−25†
2-methyl-4-chlorophenoxyacetamide	103	24	ns†
N-methyl-4-chlorophenoxyacetamide	80‖	50‡	34†
4-(2′-methyl-4′-chlorophenoxyacetamido)-benzenesulphonic acid	85‖	48	57†
2-(2′-methyl-4′-chlorophenoxyacetamido)-naphthalene-6,8-disulphonic acid	94‖	3	99†
2-(2′-methyl-4′-chlorophenoxyacetamido)-naphthalene-1-sulphonic acid	81‖	0	ns§
1-(2′-methyl-4′-chlorophenoxyacetamido)-naphthalene-8-sulphonic acid	92‖	0	ns§

* Compounds prepared at Ohio State University.

† Tributylphosphate used as co-solvent.

‡ Not entirely soluble.

§ Not sufficiently soluble for testing.

‖ Tested as NH_4 salt after addition of sufficient NH_4OH to effect solution.

¶ Tested after addition of sufficient HCl to effect solution.

TABLE 1—*Continued*

Compound	Test		
	A	B	C
2-(2′-methyl-4′-chlorophenoxyacetamido)-8-naphthol-3,6-disulphonic acid	83	0	ns§
2-methyl-4-chlorophenoxyacetic acid*	114	48	49†
2-methyl-6-chlorophenoxyacetic acid	81‖	0	ns§
2-methyl-4-chlorophenoxyacetic anhydride	101	0	58†
2-methyl-4-chlorophenoxyacetyl chloride	95	34‡	59†
2-methyl-4-fluorophenoxyacetic acid*	107	100	123†
N-methyl-2,4,5-trichlorophenoxyacetamide	103	70	ns§
2-nitro-2-methylpropyl 2′,4′-dichlorophenoxyacetate*	99	103	82†
2-nitro-2-methylpropyl 2′-methyl-4′-chlorophenoxyacetate*	84¶	32‡	132†
phenyl chloroacetate	102	39	20†
phenyl 2-methyl-4-chlorophenoxyacetate	83	62‡	−36†
isopropyl 2-methyl-4-chlorophenoxyacetate*	99‖	0‡	155
2-(2′,4′,5′-trichlorophenoxyacetamido-2-(hydroxymethyl)-1,3-propanediol	94	247	ns§
2,4,5-trichlorophenoxyacetic N-di-(2′-hydroxyethyl)-amide	81¶	0‡	120†
2,4,5-trichlorophenoxyacetic piperidide	99	56	ns§
2,4,5-trichlorophenoxyaceto-3′-chloroanilide	81	68	225†
2,4,5-trichlorophenoxyaceto-2′,4′-dimethylanilide	88	18	230†
2,4,5-trichlorophenoxyaceto-4′-ethoxyanilide	95¶	49	ns§
2,4,5-trichlorophenoxyaceto-4′-methylanilide	94	32	143†
2,4,5-trichlorophenoxyaceto-2′,4′,6′-trichloroanilide	102‖	68	217†
3-(trifluoromethyl)-phenoxyacetic acid*	94	47	−134†
N-(tris-[hydroxymethyl]-methyl)-N-(beta-hydroxy, gamma-(tris-[hydroxymethyl]-methylamino)-propyl 2,4-dichlorophenoxyacetamide, hydrochloride	86	0	14†

TABLE 2

ACTIVITIES OF COMPOUNDS: GROUP II

Compounds possessing 50–79% of the activity of 2,4-dichlorophenoxyacetic acid in corn-germination test (Test A), listed in alphabetical order. Results of kidney-bean single-droplet water test (Test B) and kidney-bean single-droplet oil test (Test C) similarly expressed as percentage of activity of 2,4-dichlorophenoxyacetic acid in same tests.

COMPOUND	TEST		
	A	B	C
beta-aminoethanol bis-(4-chlorophenoxyacetate)*	65‖	25‡	114†
4-bromophenoxyacetic acid*	50	70	50†
O-(2-carboxymethoxy-3-methyl-5-bromobenzoyl)-glycolic acid	65	64‡	5†
O-(2-carboxymethoxy-3-methyl-5-nitrobenzoyl)-glycolic acid	57‖	20	15†
monocapryl acid orthophosphate	69‖		
2-chloro-4-tertiary-butylphenoxyacetic acid*	52	50	0†
2-chloro-4-iodophenoxyacetic acid*	70	7‡	20†
alpha-chloronaphthyl-acetic acid (mixture), ammonium salt*	54		
2-(4′-chlorophenoxyacetamido)-naphthalene-1-sulphonic acid	62	2	ns§
1-(4′-chlorophenoxyacetamido)-naphthalene-4-sulphonic acid	59	28	ns§
1-(4′-chlorophenoxyacetamido)-naphthalene-8-sulphonic acid	65	46	ns§
1-(4′-chlorophenoxyacetamido)-8-naphthol-3,6-disulphonic acid	67	91	ns§
4-chlorophenoxyacetic N,N-di-(2′-hydroxyethyl)-amide	58¶	51‡	ns§
4-chlorophenoxyacetyl chloride	74	42	144†
2-(4′-chlorophenoxyacetamido)-2-(hydroxymethyl)-1,3-propanediol	72	114	59†
gamma-(4-chlorophenoxy)-butyric acid*	71	113	112†
S-(4-chlorophenyl)-thioglycolic acid	56	10	126†
crotyl 4-chlorophenoxyacetate*	78¶		108†
2,4-dibromophenoxyacetic acid*	76	94	53†
alpha, beta-dibromo-gamma-phenylpropionylchloride	57‖	10‡	ns§
3,5-dichloro-2-bromobenzoic acid	57	144	40†
2,4-dichloro-5-bromophenoxyacetic acid*	74‖	61	35
2,4-dichlorophenoxyacetic piperidide	71‖	8	25†
1-(2′,4′-dichlorophenoxyacetamido)-naphthalene-4-sulphonic acid	76	43	ns§
2,4-dichlorophenoxyacetonitrile	61	35	32†
N′-(2,4-dichlorophenoxyacetyl)-betaine hydrazide, hydrochloride	61	0	ns§
N-2,4-dichlorophenoxyacetyl diethylamine	55‖	57	50†
N-2,4-dichlorophenoxyacetyl methylamine	64‖	71	ns§
gamma-(2,4-dichlorophenoxy)-butyric acid, ammonium salt*	79		
2,4-dichlorophenylglycine*	60	6	20†
3-(2,5-dichlorophenyl)-thioglycolic acid chloride	61¶	10‡	0†
2,2-dimethyl-1,3-dioxolane-4-methanyl 4-chlorophenoxyacetate*	72	91	126
2-(2,4-dimethylphenoxy)-propionic acid*	65	0	−161†
3,5-dimethylpyrazole	50	0	0†
ethyl 3-hydroxy-2-naphthoate	52‖	0‡	57†
ethyl 2-methyl-4,6-dichlorophenoxyacetate	72	35‡	25†
2-hydroxy-3-carboxy-5-bromotoluene	55‖	0	18†
2-hydroxy-3-carboxy-5-iodotoluene	66‖	0	24†
beta-hydroxyethyl 4-chlorophenoxyacetate*	74	159	86
N-(2-hydroxyethyl)-alpha-(2′,4′-dichlorophenoxy)-acetamide*	65	0	40†
N-(2-hydroxyethyl)-alpha-(2′-methyl-4′-chlorophenoxy)-acetamide*	64	0	37†
beta-hydroxyethyl 2-methyl-4-chlorophenoxyacetate*	76	0	38
2-hydroxy-3-methylbenzoic acid	74	27	14†
2-hydroxy-5-nitrobenzoic acid	50	38	63†
2-methyl-4-bromo-6-carboxyphenoxyacetic acid	57	4	15†
3-methyl-4-chlorophenoxyacetamide	62‖	23‡	25†

* Compounds prepared at Ohio State University.

† Tributylphosphate used as co-solvent.

‡ Not entirely soluble.

§ Not sufficiently soluble for testing.

‖ Tested as NH_4 salt after addition of sufficient NH_4OH to effect solution.

¶ Tested after addition of sufficient HCl to effect solution.

TABLE 2—*Continued*

Compound	Test		
	A	B	C
methyl 4-chlorophenoxyacetate*	74	91	83†
2-methyl-5-chlorophenoxyacetic acid	70	0	79†
3-methyl-4-chlorophenoxyacetic acid*	60	0	50†
2-methyl-4-chlorophenoxyacetic diethanolamide	63¶	2‡	63†
3-methyl-4-chlorophenoxyacetyl chloride	62¶	30‡	47†
methyl 2,4-dibromophenoxyacetate*	77	108	250†
methyl 2,4-dimethylphenoxyacetate*	56	0	−10†
2-methylphenoxyacetyl chloride	67¶	0	18†
phenyl 4-chlorophenoxyacetate	65‖	97‡	240†
phenyl 2,4-dichlorophenoxyacetate	77	68‡	121†
2-(normal-propyl)-4-chlorophenoxyacetamide	72	0‡	−175†
2,4,5-trichlorophenoxyacetanilide	58	30	75†
2,4,5-trichlorophenoxyacetonitrile	54¶	0‡	ns§
N-(2,4,5-trichlorophenoxyacetyl)-di-(tris-[hydroxymethyl]-methyl-aminomethyl)-carbinol, hydrochloride	75	21	ns§

TABLE 3

ACTIVITIES OF COMPOUNDS: GROUP III

Compounds possessing 30–49% of the activity of 2,4-dichlorophenoxyacetic acid in corn-germination test (Test A), listed in alphabetical order. Results of kidney-bean single-droplet water test (Test B) and kidney-bean single-droplet oil test (Test C) similarly expressed as percentage of activity of 2,4-dichlorophenoxyacetic acid in same tests.

COMPOUND	TEST		
	A	B	C
4-aminoazobenzene	41¶	0‡	74†
beta-(amylamino)-ethyl diphenylacetate, hydrochloride	36¶	0	−5†
2-amyl-4-chlorophenoxyacetic acid*	34‖	0‡	−152†
isoamyl 2,4-dimethylphenoxyacetate	34‖	4	83†
beta-bromoethyl 4-chlorophenoxyacetate*	47	116	107
2-bromophenylsulphamic acid	35‖	0	ns§
N-butylamine, mercuric chloride	30¶	0	ns§
normal-butyl 3-methylphenoxyacetate	30	17	53†
cacotheline	37	0	50†
N-4-carboxyphenyl N′-3-chlorophenyl urea	36‖	8‡	ns§
chloroacetamide	33	0	15†
4-chlorobenzoyl chloride	37‖	34	.....
4-chlorophenoxyacetonitrile	45	17	84†
1-(4-chlorophenoxy)-2,4-epoxypropane*	41‖	21	61†
4-chlorophenylacetic acid*	38	22	−12†
N-(4-chlorophenyl)-glycine	34	0	−97
S-(4-chlorophenyl)-thioglycolic acid chloride	46	19	55†
S-(4-chlorophenyl)-thioglycolic butylamide	34	0‡	25†
2-cyanomethyl-4-chlorophenoxyacetic acid*	46	0	38†
N,N-(cyclopentamethylene)-dithiocarbamic acid, ammonium salt	31‖	0	37†
3,5-dibromo-2-aminobenzoic acid	33‖	0‡	35†
2,5-dichloro-aminobenzene, mercuri-chloride salt	33	0	−29†
2,4-dichloro-5-aminophenoxyacetic acid*	38	28	10†
2,4-dichlorocinnamic acid*	43‖	0‡	22†
2,4-dichloro-6-methylphenoxyacetamide	34	0‡	12
2,4-dichloro-5-nitrophenoxyacetic acid*	46	0	−10†
2,4-dichlorophenoxyacetic diethanolamide	49	41	93†
S-(2,5-dichlorophenyl)-thioglycolic acid	36	0‡	−10†
N,N-di-(alpha-hydroxy-beta,beta,beta-trichloroethyl)-urea	45‖	0	ns§
3,4-dimethylphenol	36	26	−4†
2,4-dimethylphenoxyacetic acid*	42	1	38†
3,4-dimethylphenoxyacetic acid*	38	0	83†
2,4-dimethylphenoxyacetyl chloride	30	35	48†
S-(2,4-dinitrophenyl)-thioglycolic acid	44	0	−81†
N,N-di-(tris-[hydroxy-methyl]-methyl)-ethylene diamine, dihydrochloride	48	0	34†
ethyl 2-chloromethyl-4-chlorophenoxyacetate*	36	0‡	39
2-ethyl-4-chlorophenoxyacetic acid*	43	39	45†
ethyl S-(4-chlorophenyl)-thioglycolate	48¶	10	−97†
2-hydroxy-3-carboxy-5-chlorotoluene	31‖	10	18†
4-hydroxy-3,5-dibromobenzoic acid	40	0	−15†
beta-hydroxyethyl 2,4-dichlorophenyl ether*	40	28	44
N4-(iodoacetyl)-sulphanilamide	37¶	0	ns§
beta-methyl beta-(butylamino)-propyl 4-hexyloxybenzoate, hydrochloride	46¶	.....	75†
2-methyl-4-chloro-6-carboxyphenoxyacetic acid	40‖	0‡	15†

* Compounds prepared at Ohio State University.

† Tributylphosphate used as co-solvent.

‡ Not entirely soluble.

§ Not sufficiently soluble for testing.

‖ Tested as NH_4 salt after addition of sufficient NH_4OH to effect solution.

¶ Tested after addition of sufficient HCl to effect solution.

TABLE 3—*Continued*

COMPOUND	TEST		
	A	B	C
methyl 2-chlorophenoxyacetate*	32	0	61†
1-(2-methyl-4-chlorophenoxy)-2,3-epoxypropane*	33¶	0	0†
methyl 2,4-dichlorophenoxyacetate*	44	100‡	108
2-methylphenoxyacetic acid	32	0	ns§
4-nitrobenzoyl chloride	42‖	33	16†
mono-octyl acid orthophosphate	45‖	.....	.....
beta-(isopropylamino)-ethyl 2-butoxybenzoate hydrochloride	32	0	63†
normal propyl 2-methyl-4-chlorophenoxyacetate*	45‖	10‡	80
isopropyl N-phenyl carbamate*	46	16	90†
beta-pyridinesulphonic acid, barium salt	33	0	−19†
sulphamerazine	38	29	.....
2,3,5-tribromobenzoic acid	41	31	24†
2,3,5-trichlorobenzoic acid	49‖	17	46†
N-(beta,beta,beta-trichloro-alpha-hydroxyethyl)-urea	32	34	62†
2,4,6-trichlorophenoxyacetic acid*	35‖	24	62†
2,4,5-trichlorophenoxyacetic 2′-nitroanilide	36	10	.....
2,4,6-trichlorophenyl N-phenyl carbamate	49‖	64	3†
S-(2,4,5-trichlorophenyl)-thioglycolic amide	38	0‡	1
1-(3-trifluoromethylphenoxy)-2,3-epoxypropane*	43	12	38†
2,3,5-triiodobenzoic acid, ammonium salt*	34	55	.....
N-(tris-[hydroxymethyl'-methyl), N-(beta-hydroxy, gamma-[tris-(hydroxymethyl)-methylamino]-propyl) 4-chlorophenoxyacetamide, hydrochloride	49	129	ns§

TABLE 4

ACTIVITIES OF COMPOUNDS: GROUP IV-A

Compounds showing less than 29% of the activity of 2,4-dichlorophenoxyacetic acid in corn-germination test (Test A) and 50% or more of the activity of 2,4-dichlorophenoxyacetic acid by either the kidney-bean single-droplet water test (Test B) or the kidney-bean single-droplet oil test (Test C), listed alphabetically.

Compound	Test		
	A	B	C
alpha-amino beta-(2,4-dichlorophenoxy)-propionamide	−38	56	−51†
alpha-amino beta-(3-nitro-4-hydroxyphenyl)-propionic acid, nitrate salt	−10	4	67†
aminotetrazole	−8	20	52†
aniline	−8	20	68†
benzyl carboxymethyl sulphone*	−8	18	55†
3-bromo-6-nitrobenzoic acid*	−3	0	50†
2-bromo-3-nitrobenzoic acid*	0	82	160†
2-bromo-3-nitrobenzoic acid, ammonium salt*	−4	99	.....
beta-bromopropionic acid	27	96	100†
beta-(butylamino)-ethyl 4-butoxybenzoate, hydrochloride	−1¶	0	94†
beta-(isobutylamino)-ethyl 4-butoxybenzoate, hydrochloride	−27¶	0	63†
beta-(butylamino)-ethyl 4-ethoxybenzoate, hydrochloride	0	.....	75†
beta-(butylamino)-ethyl 4-methoxybenzoate, hydrochloride	−20	.....	88†
camphoroxime	−8	1	110†
N4-(carbo-beta-chloro-ethoxy)-sulphanilamide	−17	0	83†
2-carbomethyl-4-chlorophenoxyacetic acid*	15	0	50†
2-carboxy-4-chlorophenoxyacetic acid	−24	0	59†
2-carboxy-6-methylphenoxyacetic acid	10	7	50†
2-carboxyphenoxyacetic acid	−16	0	82†
2-carboxymethoxy-3,5-dichlorobenzoyl glycolic acid	6	21	55†
chloroacetic acid	−22	41	70†
2-chloroaniline	−25	12	59†
3-chloroaniline	−32	0	82†
4-chloroaniline	−30	0	75†
4-chlorobenzylmercaptan*	9	12‡	55†
4-chlorobenzenesulphonyl chloride	4‖	0	69†
4-chlorobenzyl-isothiourea, hydrochloride*	10	19	72†
4-chloromandelic acid*	29	32	53†
2-chloro-4-methylphenoxyacetic acid*	11	0	70†
2-chloro-3-nitrobenzoic acid*	−18	84	96†
2-chloro-5-nitrobenzoic acid*	−3	0	100†
2-chlorophenoxyacetic acid*	29	21	66
2-(2′-chlorophenyl)-phenoxyacetic acid*	−6	0	96†
4-chlorothiophenol	−6	0	75†
diazoaminobenzene	14¶	0‡	78
2,4-dibromophenol	1	40	120
dichloroacetic acid	−38	0	56†
2,4-dichloroaniline	−11	0	85†
2,5-dichloroaniline	−23	27	61†
2,4-dichlorobenzyl carboxymethyl sulphone*	29	4	61†
2,4-dichlorobenzoic acid*	−1	0	80†
2,4-dichlorobenzyl isothiourea, hydrochloride*	23	0	50†
2,4-dichloro-6-carboxyphenoxyacetic acid	1	14	83†
2,6-dichloro-4-nitrophenoxyacetic acid*	3	13	50†
2,4-dichlorophenyl N-phenylcarbamate	1	0	78†
2,5-dichlorophenylsulphamic acid	28‖	0	50†

* Compounds prepared at Ohio State University.

† Tributylphosphate used as co-solvent.

‡ Not entirely soluble.

§ Not sufficiently soluble for testing.

‖ Tested as NH_4 salt after addition of sufficient NH_4OH to effect solution.

¶ Tested after addition of sufficient HCl to effect solution.

TABLE 4—*Continued*

Compound	Test		
	A	B	C
2,4-dihydroxypyrimidine	−28	68‡	ns§
2,4-dimethylphenol	14	9	64†
2,4-dinitrophenylacetic acid	−25	0	75†
N,N-di-(tris-[hydroxymethyl]-methyl) hexamethylenediamine, dihydrochloride	−23	0	75†
3-ethoxy-2-naphthoic acid	−27‖	0	52†
beta-(ethylamino)-butyl 4-ethoxybenzoate, hydrochloride	−19	9	56†
ethyl carbamate	−44	0	63†
ethyl beta-methyl-beta-(4-chlorophenyl)-glycidate*	26‖	0‡	84
3-ethyl-4-methyl-pyridine	−2	11	138
ethyl 2-propyl-4-chlorophenoxyacetate*	14	0‡	60†
2-fluorophenoxyacetic acid*	−51	8‡	72†
2-hydroxy-3-bromo-5-chlorobenzoic acid	23‖	20	75†
2-hydroxy-3-carboxy-5-nitrotoluene	−8	0	217†
N-(2-hydroxy-3-chloropropyl)-4-toluidine	17¶	0	74†
2-hydroxy-3,5-dinitrobenzoic acid	17	0	98†
4-iodobenzoic acid	0‖	0	100†
2-methoxyphenol	−4	0	76†
4-methoxyphenol	−22	54	.....
beta-methyl, (beta-[amylamino]-propyl)-diphenylacetate, hydrochloride	−11¶	0	54†
2-methyl-5-chlorophenol	−15	0	52†
2-methyl-6-chlorophenol	−10	0	77†
2-methyl-4-chlorophenoxyfumaric acid*	7‖	0	108†
methyl N-3-chlorophenylcarbamate	18	0	57†
2-methyl-4,6-dichlorophenol	−2	0	100†
beta-methyl beta-(hexylamino)-propyl 4-ethoxybenzoate, hydrochloride	0¶	0	75†
methyl 2-methyl-6-chlorophenoxyacetate	14	33	62
4-methylphenoxyacetic acid	11	0	75†
methyl N-phenylthiocarbamate	11	61	.....
S-(2-methylphenyl)-thioglycolic acid	20	24	65†
4-methyl-4-trichloromethyl-2,5-cyclohexadien-1-one-O-carboxymethyl oxime*	−1	0	50†
2-nitrobutyl N-phenylcarbamate	−9	18	125†
1-phenyl-3-methyl-5-pyrazolone	−33	54	.....
phthalic acid	25	0	55†
alpha-pinene	−13	0	89†
beta-(isopropylamino)-ethyl 4-butoxybenzoate, hydrochloride	−12	0	88
2-propyl-4-chlorophenoxyacetic acid*	11	23	67†
isopropyl 2,4-dimethylphenoxyacetate	24‖	14	72†
isopropyl 2-methyl-6-chlorophenoxyacetate	22	0	58
3-(normal propyl)-2-naphthoic acid	10‖	0‡	63†
isopropyl 2-propyl-4-chlorophenoxyacetate*	25‖	0‡	108
trichloroacetamide	−6	4	56†
trichloroacetic acid	−38	0	55†
trichloroacetyl chloride	−30	.....	50†
2,4,5-trichlorobenzenesulphonamide	−7¶	0‡	61†
3,4,5-trihydroxybenzoic acid	−4	54	.....
tris-(hydroxymethyl)-methyl beta,gamma-dibromo propylamine, hydrobromide	−5	0	88†
salicylic acid	26	54	.....

TABLE 5

ACTIVITIES OF COMPOUNDS: GROUP IV-B

Compounds insufficiently soluble in water for corn-germination test (Test A) but exhibiting 50% or more of the activity of 2,4-dichlorophenoxyacetic acid by either kidney-bean single-droplet water test (Test B) or kidney-bean single-droplet oil test (Test C), listed alphabetically.

COMPOUND	TEST	
	B	C
allyl 4-chlorophenoxyacetate*	147‡	94
allyl 2,4-dichlorophenoxyacetate*	76‡	85
2-aminonaphthoic acid	0	66†
normal amyl 2,4-dichlorophenoxyacetate*	88‡	81
isoamyl 2,4-dichlorophenoxyacetate*	38‡	85
normal amyl N-alpha-naphthyl carbamate	13‡	75†
4,4′-(bis-chlorophenyl)-trichloromethyl methane	18‡	72†
1,1′-(bis-beta-naphthol)-phenyl methane	20‡	67†
2-bromo-3,5-dichlorobenzamide	0‡	89†
2-bromo-3,5-dichlorobenzanilide	7	89†
2-bromo-3,5-dichlorobenzo-2′-bromoanilide	0‡	72†
2-bromo-3,5-dichlorobenzo-3′-bromoanilide	0‡	52†
2-bromo-3,5-dichlorobenzo-4′-bromoanilide	13‡	78†
2-bromo-3,5-dichlorobenzo-3′-chloroanilide	1	67†
2-bromo-3,5-dichlorobenzo-2′,4′-dichloroanilide	19‡	100†
2-bromo-3,5-dichlorobenzo-3′-toluidide	20‡	61†
2-bromo-3,5-dichlorobenzoyl chloride	63‡	110†
beta-bromoethyl 2,4-dibromophenoxyacetate*	59	100
beta-bromoethyl 2,4-dichlorophenoxyacetate*	126‡	117
4-bromophenoxyacetamide	139	ns§
N-3-bromophenyl N′-2-chlorophenyl urea	0‡	59†
N-3-bromophenyl N′3-chlorophenyl urea	0‡	60†
butyl 2,4-dichlorophenoxyacetate*	50	111
isobutyl 2,4-dichlorophenoxyacetate*	133‡	64
N-carbethoxy N′-3-chlorophenyl urea	1‡	77†
beta-chloroethyl 4-chlorophenoxyacetate*	117	97†
beta-chloroethyl 2,4-dibromophenoxyacetate*	57	77†
beta-chloroethyl 2,4-dichlorophenoxyacetate*	49	112†
beta-chloroethyl 2-methyl-4-chlorophenoxyacetate*	15	99†
beta-chloroethyl N-(alpha-naphthyl)-carbamate	8‡	67†
beta-chloroethyl N-phenyl carbamate	19	75†
4-chlorophenoxyaceto-4-anisidide	0‡	81†
4-chlorophenoxyaceto-2-bromoanilide	21‡	104†
4-chlorophenoxyaceto-3-bromoanilide	88‡	18†
4-chlorophenoxyaceto-4-bromoanilide	35‡	94†
4-chlorophenoxyaceto-2-chloroanilide	54‡	.....
4-chlorophenoxyaceto-3-chloroanilide	99‡	99†
4-chlorophenoxyaceto-2′,4′-dimethylanilide	0‡	60†
4-chlorophenoxyaceto-4′-ethoxyanilide	0‡	109†
N′-(4-chlorophenoxyacetyl) N²-phenyl hydrazine	55‡	86†
4-chlorophenoxyaceto-4′-iodoanilide	36‡	75†
4-chlorophenoxyaceto-3′-nitroanilide	0‡	108†
4-chlorophenoxyaceto-4′-toluidide	64‡	155†
4-chlorophenoxyaceto-4′-xenylamide	0‡	89†
gamma-(4-chlorophenoxy)-butyronitrile*	10	62†
4-chlorophenyl 4′chlorophenoxyacetate	136‡	85†
N-4-chlorophenyl N′-2-chlorophenyl urea	43‡	71†
4-chlorophenyl 2′,4′-dichlorophenoxyacetate	83	92†

* Compounds prepared at Ohio State University.

† Tributylphosphate used as co-solvent.

‡ Not entirely soluble.

§ Not sufficiently soluble for testing.

TABLE 5—*Continued*

Compound	Test	
	B	C
N-3-chlorophenyl N′,N′-(cyclopentamethylene) urea	55‡	64†
N-3-chlorophenyl N′-phenyl urea	0‡	64†
S-(4-chlorophenyl)-thioglycollic 2′-bromoanilide	0‡	60†
S-(4-chlorophenyl)-thioglycollic 3′-bromoanilide	29‡	70†
4-chlorophenyl 2′,4′,5′-trichlorophenoxyacetate	69‡	185†
2,6-dibromobenzoquinone-4-chlorimide	0	63†
2,4-dichlorobenzylsulphonyl chloride*	28‡	107†
1,3-di-(4-chlorophenoxyacetamido)-benzene	0	75†
1,4-di-(4-chlorophenoxyacetamido)-benzene	0	62†
4,4′-di-(4-chlorophenoxyacetamido)-biphenyl	0	50†
2,4-di-(4-chlorophenoxyacetamido)-toluene	0	88†
2,4-dichlorophenoxyacetanilide	88‡	164†
2,4-dichlorophenoxyacetic beta-aminoethylamide	132‡	ns§
2,4-dichlorophenoxyaceto-4′-anisidide	0‡	136†
2,4-dichlorophenoxyaceto-2′,5′-dichloroanilide	0‡	90†
2,4-dichlorophenoxyaceto-2′,4′-dimethylanilide	37‡	113†
N′-(2,4-dichlorophenoxyacetyl) N²-(2′,4′-dinitrophenyl) hydrazine	57‡	ns§
2,4-dichlorophenoxyacetic hydrazide	134‡	ns§
2,4-dichlorophenoxyaceto-beta-naphthalide	28‡	71†
2,4-dichlorophenoxyaceto-4′-toluidide	82‡	41†
2,4-dichlorophenoxyaceto-2′-xenylamide	54‡	75†
4-(2,4-dichlorophenoxyacetamido)-azobenzene	18‡	81†
2,4-dichlorophenoxyacetyl aminoguanidine	73‡	17†
2,4-dichlorophenoxyacetyl bromide	57†	−17†
2,4-dichlorophenoxyacetyl (dimethyl, methylol)-methylamide	7‡	67†
S-(2,4-dichlorophenoxyacetyl) isothiourea	72‡	.....
2,4-dichlorophenoxyacetyl methyl-isothiourea	56‡	ns§
gamma-(2,4-dichlorophenoxy)-butyric acid*	96	20†
gamma-(2,4-dichlorophenoxy)-butyronitrile*	3	64†
2,4-dichlorophenyl 4′-chlorophenoxyacetate	132‡	87†
2,4-dichlorophenyl 2′,4′-dichlorophenoxyacetate	68‡	112†
N-(2,5-dichlorophenyl) N′-phenyl urea	18‡	66†
S-(2,5-dichlorophenyl)-thioglycolic amide	0‡	60†
4,4′-di-(2,4-dichlorophenoxyacetamide)-biphenyl	0	52†
1,4-di-(2,4-dimethylphenoxyacetamide)-benzene	0‡	90†
2,4-di-(2,4-dimethylphenoxyacetamide)-toluene	0‡	140
2,4-dichlorophenyl-2′,4′,5′-trichlorophenoxyacetate	113	ns§
2,4-dichloropropyl 4′-chlorophenoxyacetate*	126‡	229
beta,gamma-dichloropropyl 2′,4′-dibromophenoxyacetate*	79‡	74
2,3-dichloropropyl 2′,4′-dichlorophenoxyacetate*	130‡	70
beta-diethylaminoethyl 2,3,5-triiodobenzoate*	18	50†
3,3′-dimethyl-4,4′-(4-chlorophenoxyacetamide)-biphenyl	0	81†
3,3′-dimethyl-4,4′-(2-methylphenoxyacetamido)-biphenyl	0	62†
1,3-di-(2-methylphenoxyacetamide)-benzene	0	69†
1,4-di-(2-methylphenoxyacetamide)-benzene	0	88†
4,4′-di-(2-methylphenoxyacetamide)-biphenyl	0	81†
4,4′-(2,4-dimethylphenoxy-acetamide)-diphenyl	0‡	90‡
N-4-ethoxyphenyl N′-phenyl urea	52‡	86†
ethyl 2-bromo-3,5-dichlorobenzoate	0‡	85†
ethyl 4-bromophenoxyacetate*	90	80†
ethyl 4-chlorophenoxyacetate*	88	150†
2-ethylhexyl 2′,4′-dichlorophenoxyacetate*	55	72
methallyl 4-chlorophenoxyacetate*	.....	107†
2-methoxy-4-methylphenyl N-(alpha-naphthyl)-carbamate	0‡	105†
methyl 2-bromo-3-nitrobenzoate*	90	75†
4-(2-methyl-4-chlorophenoxyacetamido)-azobenzene	0‡	67†
2-methyl-6-chlorophenoxyaceto-2′,5′-dichloroanilide	0	57†
2-methyl-4-chlorophenyl 2′,4′-dichlorophenoxyacetate	25‡	100†

TABLE 5—*Continued*

Compound	Test	
	B	C
1-methyl-2,4-di-(2,4-dichlorophenoxyacetamide)-benzene	0	65†
methyl N-4-nitrophenylcarbamate	9‡	53†
methyl 2,4,5-trichlorophenoxyacetate	83	200†
1-(betanaphthyl) N-piperidylphenyl-methane	31‡	59†
2-nitrobutyl 2,4,5-trichlorophenoxyacetate	88	390†
4-nitro-dimethylaniline	11‡	105†
normal-octyl 2,4-dichlorophenoxyacetate*	92‡	88
pentachlorophenyl 2,4,5-trichlorophenoxyacetate	3‡	210†
N-phenyl-N′,N′-cyclopentamethylene urea	63	36†
phenyl N-phenylcarbamate	0	59†
phenyl 2,4,5-trichlorophenoyxacetate	61‡	173†
isopropyl 2,4-dichlorophenoxyacetate*	94‡	68
3-isopropoxy-2-naphthoic acid	0	59†
N,N′-di-(3-tolyl) urea	0‡	85†
2,4,5-tribromo-3,5-dimethylphenoxyacetic acid	0‡	74†
2,4,6-tribromophenylacetate	0	71†
2,4,5-trichlorobenzamide	64‡	ns§
trichloroethyl 2,4-dibromophenoxyacetate*	59‡	90
beta-trichloroethyl 2,4-dichlorophenoxyacetate*	140‡	62
2,4,5-trichlorophenoxyacetic acid*	93	230†
2-(2′,4′,5′-trichlorophenoxyacetamido)-anthraquinone	51‡	175†
2,4,5-trichlorophenoxyaceto-4′-bromoanilide	51‡	83†
2,4,5-trichlorophenoxyaceto-4′-methoxyanilide	37‡	128†
2,4,5-trichlorophenoxyaceto-beta-naphthalide	0‡	218†
2,4,6-trichlorophenoxyaceto-4′-sulphonaphthalide	45‡	57†
2,4,5-trichlorophenoxyaceto-3′-toluidide	11‡	130†
2,4,5-trichlorophenoxyacetyl chloride	117‡	207†
N′-(2,4,5-trichlorophenoxyacetyl)-para-nitrophenylhydrazine	75‡	ns§
2,4,6-trichlorophenyl 4′-chlorophenoxyacetate	66‡	69†
2,4,6-trichlorophenyl 2′,4′-dichlorophenoxyacetate	85‡	46†
2,4,6-trichlorophenyl 2′,4′,5′-trichlorophenoxyacetate	77	190†
N-(3-[trifluoromethyl]-phenyl)-alpha-(4-chlorophenoxy)-acetamide	3‡	51†
N-(3-[trifluoromethyl]-phenyl)-alpha-(2,4,5-trichlorophloro-phenoxy)-acetamide*	11‡	70†
2,3,5-triiodobenzoic acid*	51	49†
2,3,5-triiodobenzoyl chloride	0	106†
1-(tris-[hydroxymethyl]-methylamino) 2,4-dinitrobenzene	0	59†
N-(para-xenyl)-2,4-dichlorophenoxyacetamide	61‡	ns§

TABLE 6

ACTIVITIES OF COMPOUNDS: GROUP IV-C

Other compounds tested in comparison with 2,4-dichlorophenoxyacetic acid by the corn-germination test (Test A), single-droplet water test (Test B), and single-droplet oil test (Test C), listed alphabetically.

COMPOUND	TEST		
	A	B	C
2-acetoxyl-ethyl N-(alpha-naphthyl)-carbamate	.....	39‡	.....
2-acetoxyl-ethyl N-phenylcarbamate	.....	15‡	.....
2-acetyl-4-chlorophenoxyacetic acid*	6	0	−20†
2-allyl-4-chlorophenoxyacetic acid*	11	0	−21†
allyl N-(alpha-naphthyl)-carbamate	ns§	0‡	2
allyl N-phenylcarbamate	.....	8	36
allyl 4-tolyl sulphone*	8	0	−47
1-aminoanthraquinone	ns§	0	ns§
2-aminoanthraquinone	ns§	0	ns§
4-aminobenzyl tris-(hydroxymethyl)-methylamine, dihydrochloride	8	0	ns§
2-amino-3,5-dichlorobenzoic acid	−17	18	48
2-amino-ethylsulphuric acid	14	27	ns§
1-amino-8-naphthol-3,6-disulphonic acid	2	0	0†
1-amino-2-naphthol-4-sulphonic acid	− 1‖	0	ns§
4-aminophenol	13	47	.....
2-aminophenoxyacetic acid	−41	7	ns§
4-aminophenylacetic acid	23	0‡	ns§
2-aminopyridine	19	0	−50†
2-aminothiazole	−20	0	21†
beta-(amyl-amino)-ethyl 4-butoxy-benzoate, hydrochloride	5	.....	25†
isoamylformate	17	47	42
normal-amyl 2-methylphenoxyacetate	22¶	0‡	35†
isoamyl N-(alpha-naphthyl)-carbamate	ns§	0	ns§
4-tertiary-amylphenol	5	9	.....
normal-amyl N-phenylcarbamate	ns§	11‡	−46
isoamyl N-phenylcarbamate	ns§	0‡	− 1
4-arsenophenoxyacetic acid	22	9	28§
benzoic acid	20	33	21†
4-(benzylamino)-phenol, hydrochloride	−23¶	0	ns§
benzyl normal-butyl sulphone*	− 6	7	ns§
benzyl carboalloxymethyl sulphone*	11	13	14
benzyl carbomethoxymethyl sulphone*	− 3	0	− 5†
N-benzyl-N,N′-(di-tris-[hydroxy-methyl]-methyl) 2-hydroxy-1,3-diaminopropane	− 7¶	.....	0†
benzyl ethyl sulphone*	− 4	0	ns§
benzyl methyl sulphone*	− 1	0	ns§
benzyl 4-tolyl sulphone*	ns§	0‡	ns§
benzyl tris-(hydroxy-methyl)-methyl amine	− 1¶	0	.....
1,3-bis-(tris-[hydroxy-methyl])-methylamino-2-propanol, dihydrochloride	− 4	36	ns§
2-bromobenzamide	15	25	−21†
2-bromo-benzanilide	ns§	0	5
2-bromo-benz-2,4-dichloroanilide	ns§	12‡	− 6
2-bromobenzoic acid	17	0	ns§
3-bromobenzoic acid	25	0	43†
4-bromobenzoic acid, ammonium salt	− 7	0	−145†
4-bromobenzonitrile	1	7	5†
2-bromo-4-tertiary-butyl-phenoxyacetic acid*	10	0	24†
2-bromo-3,5-dichloro-benzoic butylamide	ns§	14‡	44

* Compounds prepared at Ohio State University.

† Tributylphosphate used as co-solvent.

‡ Not entirely soluble.

§ Not sufficiently soluble for testing.

‖ Tested as NH_4 salt after addition of sufficient NH_4OH to effect solution.

¶ Tested after addition of sufficient HCl to effect solution.

TABLE 6—*Continued*

Compound	Test		
	A	B	C
2-bromo-3,5-dichloro-benzoic 4′-chloro-anilide	ns§	0‡	44
beta-bromoethyl amine	− 7	0	.....
beta-(bromoethyl) 4-ethoxy-thiolbenzoate	ns§	.....	25
beta-bromoethyl 2-methyl-4-chlorophenoxyacetate*	ns§	0‡	56
2-bromo-4-nitrobenzoic acid*	9	0	−10†
2-bromo-5-nitrobenzoic acid*	.....	17	.....
2-bromo-5-nitrobenzoic acid, ammonium salt*	.....	0	.....
3-bromo-4-nitrobenzoic acid*	18	0	0†
3-bromo-5-nitrobenzoic acid*	− 4	0	35†
4-bromophenol	6	20	−114†
2-bromophenoxyacetic acid*	0	0	38†
4′-bromophenoxyacetic 4-bromoanilide	ns§	10	ns§
4′-bromophenoxyacetic 4-chloroanilide	ns§	30‡	ns§
4′-bromophenoxyacetic 2,5-dichloroanilide	ns§	32‡	ns§
3-bromophenylammonium fluoborate	− 1	21	.....
4-bromophenylammonium fluoborate	6	6	.....
N-2-bromophenyl N′-2-chlorophenyl urea	ns§	42‡	ns§
N-4-bromophenyl N′-3-chlorophenyl urea	ns§	45‡	40
N-2-bromophenyl N′-3-chlorophenyl urea	ns§	0‡	7
N-4-bromophenyl N′-2-chlorophenyl urea	ns§	31‡	−42
N-4-bromophenyl-dithiocarbamic acid, ammonium salt	− 2	0‡	.....
4-bromophenyl N-(alpha-naphthyl)-carbamate	ns§	0	ns§
2-bromo-4-phenylphenoxyacetic acid*	− 1	29	−18†
4-bromophenyl N-phenylcarbamate	ns§	0	31
N-2-bromophenyl N′-phenyl urea	ns§	0‡	32
N-3-bromophenyl N′-phenyl urea	ns§	0‡	−11
N-4-bromophenyl N′-phenyl urea	ns§	0‡	2
3-bromophenyl sulphamic acid	12	0	ns§
N-(3-bromophenyl)-alpha, alpha, alpha, trichloro-acetamide	ns§	7‡	−23
beta-(butyl-amino)-ethyl 2-(butoxy)-benzoate, hydrochloride	− 4	0	12†
beta-(butyl-amino)-ethyl diphenyl-acetate, hydrochloride	−20¶	0	ns§
beta-(butyl-amino)-ethyl 4-heptyloxy benzoate, hydrochloride	20¶	.....	25†
beta-(butyl-amino)-ethyl 4-propoxybenzoate, hydrochloride	−18¶	.....	0†
beta-(butyl-amino)-ethyl 2-(thiobutoxy)-benzoate	7	16	19†
2-secondary-butyl-4-chlorophenoxyacetic acid*	26‖	0	34†
N-butyl-dithiocarbamic acid, mercury salt	ns§	15‡	ns§
normal-butyl N-(alpha-naphthyl)-carbamate	ns§	0‡	21
isobutyl N-(alpha-naphthyl)-carbamate	ns§	5‡	8
4-tertiary-butylphenol	−13	0	.....
normal-butyl N-phenylcarbamate	ns§	18	44
isobutyl N-phenylcarbamate	ns§	15	45
tertiary-butyl N-phenylcarbamate	ns§	43‡	ns§
N-(normal-butyl) N′-phenyl thiourea	− 4	0	46†
N-(normal-butyl)-2,4,5-trichlorophenoxy-acetamide	ns§	0	.....
4-carbethoxy-6-methoxyquinoline	−25	8	47†
N-carbethoxy N′-phenyl urea	ns§	0	ns§
alpha-(carbo-normal-butoxy)-ethyl N-(alpha-naphthyl)-carbamate	ns§	24	− 8
alpha-(carbo-isopropoxy)-ethyl N-(alpha-naphthyl)-carbamate	ns§	19	22
O-(2-carboxymethoxy-benzoyl)-glycolic acid	17	20	38†
O-(2-carboxymethoxy-3-methyl-5-chloro-benzoyl)-glycolic acid	27	0‡	23†
N-(carboxymethyl)-dithiocarbamic acid, ammonium salt	5	12‡	ns§
N-(4-carboxy-methyl-phenyl)-dithiocarbamic acid, sodium salt	0	0	ns§
2-carboxy-6-methylphenyl N-phenyl-carbamate	20‡	12‡	ns§
N-(4-carboxy-phenyl)-dithiocarbamic acid, ammonium salt	6	0	ns§
4-carboxyphenylglycine	17	0	−17†
ortho-carboxyphenyl N-(alpha-naphthyl)-carbamate	ns§	2	ns§
N-(4-carboxyphenyl) N′-(alpha-naphthyl) urea	−22	34	ns§
4-carboxyphenyl N-phenylcarbamate	ns§	0‡	ns§
S-(4-carboxyphenyl)-thioglycolic acid	−18‖	0	−19†

TABLE 6—*Continued*

Compound	Test		
	A	B	C
N^4-(beta-carboxy-propionyl)-sulphanilamide	−19‖	0	ns§
catechol	−34	0	12†
chloroacetyl chloride	− 6	3	49†
4-chloroanisole*	−31	0	− 8†
2-chlorobenzaldehyde O-carboxymethyl oxime*	4	8	10†
2-chlorobenzaldoxime*	20	1	5†
4-chlorobenzamide	4	7	ns§
4-chlorobenzenesulphonamide	ns§	0	44
4-chlorobenzoic acid	6	0	ns§
bis-(4-chlorobenzyl) disulphide*	ns§	27‡	55
S-(4-chlorobenzyl)-thioglycolic acid*	− 9	25	−15†
bis-(4-chlorobenzyl) sulphide*	ns§	18‡	36
4-chlorobenzylsulphonylacetic acid*	2	5	−15†
4-chlorocinnamic acid*	26‖	23‡	43†
highly chlorinated 1,5-dihydroxynaphthalene	ns§	0	13
beta-chloroethyl 2-propyl-4-chlorophenoxyacetate*	ns§	0‡	46
chlorohydroquinone	18	47	.....
chlorohydroquinone-O, O-diacetic acid*	1	0	ns§
4-(chloromercuri)-phenol	28‖	6	ns§
4-(chloromercuri)-phenoxyacetic acid	ns§	18‡	ns§
2-chloromethyl 4′-chlorophenoxyacetic acid*	17‖	0	38†
2-chloro-4-methyl-6-methoxyquinoline	ns§	0‡	ns§
2-chloro-4-methylquinoline	ns§	0	20
7-chloro-1-naphthoxyacetic acid*	13	16	−12†
alpha-chloronaphthylacetic acid mixt.*	ns§	12	14
4-chlorophenetole*	−8	0	−29†
1-(4′-chlorophenoxyacetamido)-naphthalene	ns§	12‡	ns§
2-(4′-chlorophenoxyacetamido)-naphthalene	ns§	4‡	ns§
4′-chlorophenoxyacetic 2,5-dichloroanilide	ns§	47‡	ns§
4-chlorophenoxyacetic diethylamide	.....	44	.....
4-chlorophenoxyacetic piperidide	17	7	ns§
4′-chlorophenoxyacetic 2-nitroanilide	ns§	14‡	ns§
4′-chlorophenoxyacetic 2,4,6-trichloroanilide	ns§	0‡	19
4-chlorophenoxy 4′-chlorophenyl acetic acid*	−19	25	0†
4-chlorophenoxyfumaric acid*	0	0	ns§
alpha-(4-chlorophenoxy)-heptylic acid*	22	0	−191†
beta-(4-chlorophenoxy)-propionic acid*	2	30	12†
beta-(4-chlorophenoxy)-propionitrile*	−38	24	24†
4-chlorophenylammonium fluoborate	10	61	.....
N-2-chlorophenyl N′-normal-butyl urea	.....	10‡	3
N-3-chlorophenyl N′-normal-butyl urea	23	5‡	20†
N-2-chlorophenyl N-4-carboxyphenyl urea	ns§	21	.....
N-(3-chloro-phenyl)-alpha-chloro-acetamide	−21	0	32†
N-(4-chloro-phenyl)-alpha-chloro-acetamide	5¶	0‡	−51†
N-3-chlorophenyl N′-2-chlorophenyl urea	ns§	24‡	−27
N-4-chlorophenyl N′-3-chlorophenyl urea	ns§	0‡	ns§
N-2-chlorophenyl N′,N′-cyclopentamethylene urea	ns§	19	23
N-4-chlorophenyl dithiocarbamic acid, ammonium salt	19	0	.....
2-chloro-1,4-phenylene-bis-(N-phenyl carbamate)	ns§	0	ns§
N-(2-chlorophenyl)-glycine	−16	7	38†
N-2-chlorophenyl N′-beta-hydroxyethyl urea	ns§	0‡	−21
N-3-chlorophenyl N′-beta-hydroxyethyl urea	ns§	23‡	−27
3-chlorophenyl isocyanate	ns§	0‡	0
N-(2-chlorophenyl) N′-(alpha-naphthyl) urea	ns§	19‡	ns§
N-(4-chlorophenyl) N′-(alpha-naphthyl) urea	ns§	0	ns§
2-(4′-chlorophenyl)-phenoxyacetic acid*	5	4	40
N-(2-chlorophenyl) N′-phenyl urea	ns§	0‡	28
N-(4-chlorophenyl) N′-phenyl urea	ns§	12‡	ns§
N-(2-chlorophenyl) N′-phenyl thiourea	ns§	0‡	ns§

TABLE 6—*Continued*

Compound	Test		
	A	B	C
N-(3-chlorophenyl) N′-phenyl thiourea	ns§	10‡	14
N-(4-chlorophenyl) N′-phenyl thiourea	ns§	0‡	22
3-chlorophenyl sulphamic acid, sodium salt	13	0	6†
4-chlorophenyl sulphamic acid	−11	0	ns§
S-(2-chlorophenyl)-thioglycolic acid	5	0	7†
S-(4-chlorophenyl)-thioglycolic amide	28	6‡	40†
S-(4-chlorophenyl)-thioglycolic anilide	ns§	32‡	40
S-(4-chlorophenyl)-thioglycolic 4′-bromoanilide	ns§	0‡	35
S-(4-chlorophenyl)-thioglycolic 4′-phenetidide	ns§	0‡	38
S-(4-chlorophenyl)-thioglycolic 3′-toluidide	ns§	23†	45
N-2-chlorophenyl urea	ns§	0†	ns§
N-3-chlorophenyl urea	ns§	0†	− 6
N,N′-di-(2-chlorophenyl) urea	ns§	28†	ns§
N,N′-di-(3-chlorophenyl) urea	ns§	0†	− 6
cinnamic amide	12	6	ns§
cinnamoyl chloride	9	27	−115†
2-cresol	− 6	0	−134
3-cresol	0	2	−20
4-cresol	−29	5	−32
4-cresoxyacetyl chloride	− 3	31	25†
cyanoacetamide	17	8	28†
2-cyclohexyl-4-chlorophenoxyacetic acid*	18‖	8†	33†
decyl carboxymethyl sulphide*	13‖	0†	−10†
normal-decyl carboxy-methyl sulphone*	10	0	−53†
di-(2-acetoxyethyl) sulphone*	−13	23	29
2,6-diaminopyridine, monohydrochloride	−24	28	ns§
2,6-dibromo-4-carboxyphenyl N-phenylcarbamate	ns§	23	35
alpha, beta-dibromo-dihydrocinnamic acid	3	6	− 5†
4,6-dibromo-1,3-dihydroxy-benzene	18	0	15†
2,6-dibromo-4-methyl-phenoxyacetic acid	7	0	14
2,4-dibromophenyl N-phenylcarbamate	ns§	0	3
alpha, beta-dibromo-gamma-phenylpropionamide	ns§	18‡	−14
di-(2-butyroxyethyl) sulphone*	− 5	0	3
2,5-dichloro-4-aminobenzenesulphonic acid	8	0	27†
2,4-dichloroanisole*	−79	0	15†
2,6-dichlorobenzeneoneindophenol, sodium salt	−28	6	0†
2,5-dichlorobenzenesulphonamide	−39¶	11	−33†
2,5-dichlorobenzenesulphonyl chloride	ns§	0‡	48
2,4-dichlorobenzyl carboxymethyl sulphide*	15	32	28†
bis-(2,4-dichlorobenzyl) disulphide*	ns§	2‡	2
2,4-dichlorobenzyl mercaptan*	ns§	0‡	23
di-(2,4-dichlorobenzyl) sulphide*	ns§	12‡	27
di-(2,4-dichlorobenzyl) sulphone*	ns§	23‡	23
5,7-dichlorocoumaran-3-one			15
2,4-dichloro-N-chloroacetanilide*	6	12	0†
2,6-dichloro-3-ethyl-4-methyl-pyridine	ns§	0	44
2,4-dichloromandelic acid*	13‖	5	43†
2,6-dichloro-4-methyl-5-ethyl-nicotinamide	−19	0	ns§
2,6-dichloro-4-methylphenoxyacetic acid*	− 1	0	50
2,4-dichloro-6-methyl-phenoxyacetyl chloride	ns§	0	44
2,4-dichloro-1-naphthoxyacetic acid*		0	−40
2,4-dichlorophenetole*	0	0	−37†
2,4-dichlorophenol	13	0	−16†
1-(2′,4′-dichlorophenoxyacetamido)-anthraquinone	ns§	45‡	ns§
2-(2′,4′-dichlorophenoxyacetamido)-anthraquinone	ns§	45‡	ns§
2,6-dichlorophenoxyacetic acid*	15	15	15†
3,5-dichlorophenoxyacetic acid*	17	0	24†
2,4-dichlorophenoxyaceto-4′-bromoanilide	ns§	6‡	20
2,4-dichlorophenoxyaceto-4′-chloroanilide	ns§	23‡	3

TABLE 6—*Continued*

Compound	Test		
	A	B	C
2,4-dichlorophenoxyaceto-4′-phenetidide	ns§	27‡	ns§
2,4-dichlorophenoxyacetic N-(beta-hydroxyethyl)-amide	ns§	37‡	ns§
2,4-dichlorophenoxyaceto-alpha-naphthalide	ns§	31‡	ns§
2,4-dichlorophenoxyaceto-2′-nitroanilide	ns§	38‡	ns§
2,4-dichlorophenoxyaceto-3′-nitroanilide	ns§	4‡	ns§
N^1-(2′,4′-dichlorophenoxyacetyl) N^2-(para-nitrophenyl) hydrazine	ns§	19‡	ns§
2,4-dichlorophenoxyacetic (2′-pyridyl)-amide	.....	.....	28
2,4-dichlorophenoxyaceto-2′,4′,6′-trichloroanilide	ns§	27‡	ns§
2-(2′,4′-dichlorophenoxyacetamido)-naphthalene-6,8-disulphonic acid	.....	36	128
N^1-(2′,4′-dichlorophenoxyacetyl) N^1-phenylsemicarbazide	ns§	21‡	ns§
2,4-dichlorophenoxy para-chlorophenylacetic acid*	4	41	6†
1-(2′,4′-dichlorophenoxy)-2,3-epoxypropane*	ns§	0	0
2,4-dichlorophenoxyfumaric acid*	−15	0	ns§
alpha-(2,4-dichlorophenoxy)-heptylic acid*	17	0‡	−107†
beta-(2,4-dichlorophenoxy)-propionic acid	−22	0	6†
alpha-(2,4-dichlorophenoxy)-stearic acid*	ns§	0	10
N-(2,4-dichlorophenyl)-alpha-chloroacetamide	9‖	0‡	22†
N-(2,5-dichlorophenyl)-alpha-chloroacetamide	ns§	0‡	22
2,4-dichlorophenyl alpha-chloroacetate	−22	22	−15†
N-2,5-dichlorophenyl chlorophenyl urea	ns§	0‡	ns§
N-2,5-dichlorophenyl N′-3′-chlorophenyl urea	ns§	0‡	ns§
N-(2,5-dichlorophenyl)-glycine*	−10	19	40†
2,4-dichlorophenyl 2′-methyl-4′-chlorophenoxyacetate	ns§	10	0
2,4-dichlorophenyl N-(alpha-naphthyl)carbamate	ns§	0‡	ns§
N-(2,4-dichlorophenyl) N′-(alpha-naphthyl) urea	ns§	27	ns§
N-(2,4-dichlorophenyl) N′-phenyl thiourea	ns§	0‡	17
N-(2,5-dichlorophenyl) N′-phenyl thiourea	ns§	0‡	−38
N-(2,4-dichlorophenyl) N′-phenyl urea	ns§	24‡	31
S-(2,4-dichlorophenyl)-thioglycolic acid	11	0	10†
S-(2,5-dichlorophenyl)-thioglycolic anilide	ns§	0‡	20
S-(2,5-dichlorophenyl)-thioglycolic 4′-anisidide	ns§	28‡	10
S-(2,5-dichlorophenyl)-thioglycolic 2′-bromoanilide	ns§	0‡	10
S-(2,5-dichlorophenyl)-thioglycolic 3′-bromoanilide	ns§	6‡	20
S-(2,5-dichlorophenyl)-thioglycolic 3′-chloroanilide	ns§	10‡	−10
S-(2,5-dichlorophenyl)-thioglycolic 2′,4′-dichloroanilide	ns§	10	28
S-(2,5-dichlorophenyl)-thioglycolic diphenylamide	ns§	9‡	20
S-(2,5-dichlorophenyl)-thioglycolic 4′-toluidide	ns§	38‡	14
2-(beta, gamma-dichloropropyl)-4-chlorophenoxyacetic acid*	−28	0	−43†
3,5-dichlorosalicylic acid	7‖	0	−106†
2,4-dichlorothiophenol	−10¶	29	26†
1,3-di-(2′,4′-dichlorophenoxyacetamido)-benzene	ns§	0	− 5
1,4-di-(2′,4′-dichlorophenoxyacetamido)-benzene	ns§	0	12
1,3-di-(2′,4′-dimethylphenoxyacetamido)-benzene	ns§	0‡	47
N-(alpha-[diethylamino]-diethylacetyl) urea	8	0	4†
N,N-diethyldithiocarbamic acid, sodium salt	− 7	8	ns§
2,6-dihydroxy-3-ethyl-4-methyl-5-carbethoxypyridine	−10	11	− 5†
2,6-dihydroxy-3-ethyl-4-methyl-5-cyanopyridine	−24	21	0†
2,6-dihydroxy-3-ethyl-4-methylpyridine	−22	0	ns§
2,6-dihydroxy-4-methyl-5-cyanopyridine, ammonium salt	2	9	ns§
1,5-dihydroxynaphthalene	ns§	39	25
2,5-dihydroxytoluene	−26	−15	0†
3,4-diiodobenzoic acid*	− 4	11	.....
2,4-diiodophenoxyacetic acid*	23	0	11†
2,4-diketo-1,2,3,4-tetrahydro-pyrimidine-6-acetic acid	13	0	ns§
2,4-dimethyl-3,5-dicarbethoxy-pyrrole	ns§	0‡	10
3,5′-dimethyl-4,4′-di-(2″,4″-dichlorophenoxyacetamido)-biphenyl	ns§	0‡	10
3,3′-dimethyl-4,4′-di-(2″,4″-dimethylphenoxyacetamido)-biphenyl	ns§	0‡	14
3,5-dimethylisoxazole	0	0	19†
2,5-dimethylphenol	5	0	−159

TABLE 6—*Continued*

Compound	Test		
	A	B	C
2,4-dimethylphenoxyacetamide	−15	0	− 4†
2,4-di-(2′-methylphenoxyacetamido)-toluene	ns§	0‡	38
1-(2′,4′-dimethylphenoxyacetamido)-2,4-dichlorobenzene	ns§	0‡	5
1-(2′,4′-dimethylphenoxyacetamido)-2,4,6-trichlorobenzene	ns§	2‡	48
2,5-dimethylphenoxyacetic acid*	− 1	8	30†
3,5-dimethylphenoxyacetic acid*	3	13	5†
2,4-dimethylphenoxyaceto-4′-anisidide	ns§	0‡	40
2,4-dimethylphenoxyaceto-4′-chloroanilide	ns§	0‡	20
2,4-dimethylphenoxyaceto-beta-naphthalide	ns§	0‡	0
2,4-dimethylphenoxyaceto-3′-toluidide	ns§	5	−32
2,4-dimethylphenoxyaceto-4′-toluidide	ns§	0‡	22
2-(2′,4′-dimethylphenoxyacetamido)-anthraquinone	ns§	0‡	− 5
1-(2′,4′-dimethylphenoxyacetamido)-naphthalene-4-sulphonic acid	−33	4	22†
N-2,4-dimethylphenyl N′-2-chlorophenyl urea	ns§	25‡	ns§
N-2,4-dimethylphenyl N′-3-chlorophenyl urea	ns§	0‡	ns§
N-(2,4-dimethylphenyl)-dithiocarbamic acid, ammonium salt	ns§	4	12
2,4-dimethylphenyl N-(alpha-naphthyl)-carbamate	ns§	0‡	ns§
2,5-dimethylphenyl N-(alpha-naphthyl)-carbamate	ns§	0‡	ns§
3,4-dimethylphenyl N-(alpha-naphthyl)-carbamate	ns§	0‡	24
N-2,4-dimethyl-phenyl N′-phenyl urea	ns§	2‡	ns§
3,5-dinitrobenzamide	12¶	0	38†
3,5-dinitrobenzoyl chloride	ns§	25	5
2,4-dinitro-1-naphthol-7-sulphonic acid	17	21	23†
2,4-dinitrophenol	13	9	24†
2,4-dinitrophenoxyacetic acid	21	0	.....
N-(2,4-dinitrophenyl)-glycine	13	9	42†
2,4-dinitrophenylhydrazine	ns§	6	18
N,N′-di-(4-nitrophenyl) urea	ns§	6	ns§
N,N-(diphenyl)-2,4-dichlorophenoxyacetamide	ns§	0‡	ns§
N,N-diphenyl N′-phenyl urea	ns§	4‡	ns§
N,N′-diphenylthiourea	ns§	18‡	5
N,N′-diphenyl urea	ns§	0‡	ns§
N,N′-di-(4-sulphamidophenyl)-oxalamide	ns§	0‡	ns§
3,6-disulpho-2-naphthyl N-phenylcarbamate, disodium salt	ns§	8‡	ns§
dithio-oxamide	5	16	25†
ethoxyacetic acid	−29	0	−100
beta-(ethylamino)-ethyl diphenyl-acetate, hydrochloride	−30	0	ns§
ethyl alpha-bromoacetoacetate	−30	13	−75
ethyl 4-bromophenylcarbonate*	−36	26	19§
ethyl 2-bromo-4-phenylphenoxyacetate*	ns§	23‡	57
ethyl 2-secondary-butyl-4-chlorophenoxyacetate*	22‖	0‡	17
ethyl 2-chlorophenoxyacetate*	7	14	−11†
ethyl alpha-(4-chlorophenoxy)-heptoate*	17‖	0‡	−17
ethyl 4-chlorophenylcarbonate*	12	16	29†
ethyl alpha-cyano-beta-methyl-gamma-ethyl-glutaconate	8‖	0	22†
ethyl 2,4-dibromophenylcarbonate*	ns§	17	31
ethyl dichloroacetate	− 3	12	0†
ethyl 2,4-dichloro-5-aminophenoxyacetate*	25	11‡	− 1†
ethyl 2,4-dichloro-5-nitrophenoxyacetate*	− 8	1	0†
ethyl alpha-(2,4-dichlorophenoxy)-heptoate*	43‖	16‡	−43
ethyl 2,4-dichlorophenylcarbonate*	ns§	35‡	35
ethyl S-(2,5-dichlorophenyl)-thioglycolate	ns§	0‡	−10
ethyl 2-methyl-6-chlorophenoxyacetate	ns§	0	31
2-(3-ethyl-4-methyl-5-cyano-6-hydroxypyridyl)-N-phenylcarbamate	ns§	0	60
2,6-(3-ethyl-4-methyl-5-cyanopyridine)-bis-N-phenylcarbamate	ns§	0	0
ethyl N-(alpha-naphthyl)-carbamate	ns§	0	.....
ethyl N-phenylcarbamate	10	0	−15†
bis-beta, beta-ethyl sulphone dilaurate*	ns§	0‡	ns§
ethyl N-(beta,beta,beta-trichloro-alpha-hydroxyethyl)-carbamate	−39	0	13†

TABLE 6—*Continued*

Compound	Test		
	A	B	C
ethyl 2,4,5-trichlorophenoxyacetate	10	0	0†
ethyl S-(2,4,5-trichlorophenyl)-thioglycolate	10	0	0†
4-formylphenyl N-(alpha-naphthyl)-carbamate	ns§	0‡	ns§
hexachlorophenol	57	18	0‡
hydrazo di-carbonamide	− 1	2	21
4-hydroxyacetanilide	6	0	ns§
4-hydroxybenzaldehyde	−71	0	−111
4-hydroxybenzenesulphonic acid, sodium salt	13	9	ns§
4-hydroxybenzoic acid	−18	31	.....
O-(2-hydroxybenzoyl)-glycolic acid	− 6	19	9†
2-hydroxy-5-bromobenzoic acid	15	0	19†
2-hydroxy-3-bromo-5-sulphobenzoic acid	−24	39	28†
2-hydroxy-5-chlorobenzoic acid	−13	20	19†
2-hydroxy-5-chloroxenyl mercuric hydroxide	ns§	0‡	ns§
2-hydroxy-3,5-dibromobenzoic acid	− 8	0	16†
2-hydroxy-3,5-diiodobenzoic acid	28	0	−13†
N-(beta-hydroxyethyl) 4-chlorophenoxyacetamide*	9	0	−34†
N-(beta-hydroxyethyl)-dithiocarbamic acid, sodium salt	17	0	ns§
N-(beta-hydroxyethyl) N′-phenyl thiourea	−57	0	−14†
N-(beta-hydroxyethyl)-S-(2,4,5-trichlorophenyl)-thioglycolic amide	10	.....	0†
2-hydroxy-3-methyl-5-bromo-benzoyl chloride	ns§	0‡	12
2-hydroxy-3-methyl-5-chloro-benzoyl chloride	ns§	0‡	15
2-hydroxy-4-methyl-6-methoxyquinoline	ns§	0	ns§
2-hydroxy-3-methyl-5-nitrobenzamide	−29	0	ns§
2-hydroxy-3-methyl-5-nitrobenzoyl chloride	1	0	15†
N-(2-hydroxyisopropyl) alpha-(2-methyl-4-chlorophenoxyl)-acetamide*	25	0	−17†
2-hydroxy-4-methylquinoline	− 8	3	ns§
2-hydroxy-3-nitrobenzoic acid	0	0	−17†
2-hydroxy-3-nitro-5-bromobenzoic acid	20	0	42†
2-hydroxy-3-nitro-5-chlorobenzoic acid	−22	17	8†
2-hydroxy-3-nitro-5-sulphobenzoic acid	−25	14	24†
4-hydroxyphenylarsonic acid	− 8	5	ns§
N-(4-hydroxyphenyl)-dithiocarbamic acid, ammonium salt	6	0	−40†
N-(4-hydroxyphenyl)-glycine	− 6	3	6†
2-hydroxypyridine	−14	0	0
beta-hydroxypyridine, sulphate	−22	27	ns§
hydroquinone	−21	0	29†
5-iodo-2-aminobenzoic acid	2	16	−102†
2-iodobenzoic acid	−33	11	29†
3-iodobenzoic acid	1	29	−70†
2-iodo-3-nitrobenzoic acid*	−34	22	25†
2-iodophenol	17	23	− 9†
N-(4-iodophenyl)-dithiocarbamic acid, ammonium salt	ns§	0	ns§
2-iodophenyl N-phenylcarbamate	ns§	0	44
2-keto-4-phenyl-5-carbethoxy-6-methyl-1,2,3,4-tetrahydropyrimidine	ns§	22	ns§
2-methoxy-4-methylphenoxyaceto-2′,5′-dichloroanilide	ns§	0	ns§
2-methoxy-4-methylphenyl N-phenylcarbamate	ns§	0	−28
2-methoxy-4-methylphenyl N-3-tolylcarbamate	ns§	0‡	ns§
2-methoxy-4-methylphenyl N-4-tolylcarbamate	ns§	0‡	ns§
4-methoxyphenylammonium fluoborate	7	22	.....
N-(4-methoxyphenyl)-dithiocarbamic acid, ammonium salt	8	0	ns§
4-methoxyphenyl N-(alpha-naphthyl)-carbamate	ns§	0‡	ns§
N-(4-methoxyphenyl) N′-phenyl urea	ns§	0‡	ns§
2-(methylamino)-benzoic acid	23	0	−15†
beta-methyl-beta-(amylamino)-propyl 4-ethoxybenzoate, hydrochloride	−11	.....	31
beta-methyl-beta-(amylamino)-propyl 4-heptyloxybenzoate, hydrochloride	17	22	0†
beta-methyl-beta-(amylamino)-propyl 4-propoxybenzoate, hydrochloride	−20¶	.....	.....

TABLE 6—*Continued*

Compound	Test		
	A	B	C
beta-methyl-beta-(butylamino)-propyl 4-amyloxybenzoate, hydrochloride	1¶	16	− 6†
beta-methyl-beta-(butylamino)-propyl diphenylacetate, hydrochloride	20¶	0	12
beta-methyl-beta-(butylamino)-propyl 4-heptyloxybenzoate, hydrochloride	8¶	.....	19†
2-methyl-6-carboxyphenyl N-(alpha-naphthyl)-carbamate	ns§	0	14
2-methyl-6-chlorophenoxyacetamide	10	0	29†
2-methyl-6-chlorophenoxyacetanilide	ns§	18	30
2-methyl-6-chlorophenoxyacetic (normal-butyl)-amide	ns§	14	7
2-methyl-6-chlorophenoxyaceto-4′-chloroanilide	ns§	0	0
2-methyl-4-chlorophenoxyacetic hydrazide	ns§	27‡	ns§
2-methyl-4-chlorophenoxyaceto-3′-toluidide	ns§	8‡	46
2-methyl-6-chlorophenoxyaceto-3′-toluidide	ns§	0‡	−15
2-methyl-6-chlorophenoxyaceto-4′-toluidide	ns§	7	−14
2-methyl-6-chlorophenoxyacetyl chloride	25	0	25†
alpha-(2-methyl-4-chlorophenoxy)-heptylic acid*	9‖	0	−34
2-methyl-4-chlorophenyl N-phenylcarbamate	− 1	0	−17
2-methyl-5-chlorophenyl N-phenylcarbamate	ns§	0	29
2-methyl-6-chlorophenyl N-phenylcarbamate	ns§	0	24
3-methyl-4-chlorophenyl N-phenylcarbamate	ns§	0	23
2-methyl-4,6-dibromophenoxycetic acid*	ns§	0	0
2-methyl-4,6-dichlorophenoxyacetic acid*	ns§	15	−41
2-methyl-4,6-dichlorophenoxyacetanilide	ns§	0‡	− 5
2-methyl-4,6-dichlorophenoxyacetic (normal-butyl)-amide	ns§	0	0
2-methyl-4,6-dichlorophenoxyaceto-para-chloroanilide	ns§	5	17
2-methyl-4,6-dichlorophenoxyaceto-2′,5′-dichloroanilide	ns§	14	20
2-methyl-4,6-dichlorophenoxyaceto-3′-toluidide	ns§	0‡	25
2-methyl-4,6-dichlorophenoxyaceto-4′-toluidide	ns§	7	−34
methyl 2-methyl-4,6-dichlorophenoxyacetate	ns§	17	30
beta-methyl-beta-(ethyl-amino)-propyl 4-butoxybenzoate, hydrochloride	−31	.....	25†
S-methyl isothiourea sulphate	10	0	ns§
methyl N-(alpha-naphthyl)-carbamate	ns§	0	− 5
2-methyl-1,4-phenylene-bis-(N-alpha-naphthylcarbamate)	ns§	0	ns§
2-methylphenoxyacetamide	28	0	24†
3-methylphenoxyacetamide	−13	13	−10†
4-methylphenoxyacetamide	6	0	24†
3-methylphenoxyacetyl chloride	9‖	26	30†
methyl N-phenylcarbamate	−17	13	46†
N-4-methylphenyl urea	8	0	ns§
beta-methyl-beta-(propylamino)-propyl 4-heptyloxybenzoate, hydrochloride	20¶	.....	.....
4-methyl-4-trichloromethyl 2-chloro-2,5-cyclohexadienone*	−13	0	35†
4-methyl-4-trichloromethyl 2-chloro-2,5-cyclohexadiene-1-one-oxime*	ns§	5‡	8
4-methyl-4-trichloromethyl 2,6-dichloro-2,5-cyclohexadienone*	ns§	12‡	0
4-methyl-4-(trichloromethyl)-5-cyclohexadiene-1-one-oxime*	−16‖	11	25†
4-methyl-4-trichloromethyl-2,5-cyclohexadienone*	22	19	20†
alpha-naphthol	−12	39	0†
beta-naphthol	2	18	39†
1,2-naphthoquinone-4-sulphonic acid, sodium salt	−21	.....	.....
beta-naphthoxyacetamide	ns§	0‡	21
alpha-naphthoxyacetic acid, ammonium salt	− 3	0	0
beta-naphthoxyacetic acid	18	6	25†
2-naphthylamine-6,8-disulphonic acid	− 7	14	ns§
2-naphthylamine-1-sulphonic acid	− 9	0	ns§
1-naphthylamine-4-sulphonic acid	0	9	ns§
1-naphthylamine-8-sulphonic acid	− 7	4	0
N-(alpha-naphthyl)-dithiocarbamic acid, ammonium salt	ns§	0‡	−11
N-(beta-naphthyl)-dithiocarbamic acid, ammonium salt	1	0‡	.....

TABLE 6—*Continued*

Compound	Test		
	A	B	C
N-(alpha-naphthyl)-glycine	−12	1	ns§
alpha-naphthyl N-(alpha-naphthyl)-carbamate	ns§	0‡	ns§
N-(beta-naphthyl) N′-(alpha-naphthyl) urea	ns§	0‡	ns§
N-alpha-naphthyl N′-phenyl urea	ns§	0‡	−14
N-beta-naphthyl N′-phenyl urea	ns§	0‡	−46
N-alpha-naphthyl urea	ns§	0‡	ns§
N,N′-di-(alpha-naphthyl) urea	ns§	26	ns§
nicotinyl chloride-hydrochloride	6	.....	10†
nicotinyl nitrile	15	.....	20†
4-nitroacetanilide	7	21	ns§
4-nitrobenzamide	7	12	ns§
4-(4′-nitrobenzamido)-benzenesulphonic acid, potassium salt	− 6	0	ns§
1-(4′-nitrobenzamido)-2-bromobenzene	ns§	0‡	ns§
1-(4′-nitrobenzamido)-3-bromobenzene	ns§	4‡	ns§
1-(4′-nitrobenzamido)-4-bromobenzene	ns§	0‡	ns§
1-(4′-nitrobenzamido)-2-chlorobenzene	ns§	0‡	ns§
1-(4′-nitrobenzamido)-3-chlorobenzene	ns§	14	−56
1-(4′-nitrobenzamido)-4-chlorobenzene	ns§	6‡	48
1-(4′-nitrobenzamido)-2,5-dichlorobenzene	ns§	0‡	42
1-(4′-nitrobenzamido)-2,4-dimethylbenzene	ns§	0‡	ns§
1-(4′-nitrobenzamido)-4-iodobenzene	ns§	0‡	ns§
1-(4′-nitrobenzamido)-4-nitrobenzene	ns§	0‡	95
3-nitrobenzenediazonium fluoborate	−29	0	ns§
4-nitrobenzenediazonium fluoborate	−15	0	ns§
4-nitrobenzoic acid	37	36	10
3-nitrobenzoic acid	1	14	.....
4-nitrobenzyl tris-(hydroxymethyl)-methyl amine	− 4	0	ns§
2-nitro-3-bromobenzoic acid*	ns§	0	43
2-nitro-4-bromobenzoic acid*	−21	0	27
3-nitro-4-bromobenzoic acid*	ns§	0	45
2-nitrophenoxyacetic acid	− 6	6	47†
4-nitrophenoxyacetic acid	13‖	0	16†
N-2-nitrophenyl N′-2-chlorophenyl urea	ns§	0‡	ns§
N-3-nitrophenyl N′-2-chlorophenyl urea	ns§	0‡	ns§
N-4-nitrophenyl N′-2-chlorophenyl urea	ns§	0‡	−42
N-(4-nitrophenyl)-dithiocarbamic acid, ammonium salt	3	0	27†
N-2-nitrophenyl N′-phenyl urea	ns§	0‡	ns§
N-3-nitrophenyl N′-phenyl urea	ns§	0‡	20
N-4-nitrophenyl N′-phenyl urea	ns§	0‡	ns§
S-(4-nitrophenyl)-thioglycolic acid	−24	4	ns§
5-nitro-2-pyridyloxyacetic acid*	1	27	ns§
4-nitroso-N,N-dimethylaniline, hydrochloride	−48	3	ns§
4-nitrotoluene	−16	0	−23
octadecyl 2,4-dichlorophenoxyacetate*	ns§	69‡	61
normal-octyl carbomethoxymethyl sulphone*	− 7	0	34
octyl carboxypropyl sulphide*	1‖	0‡	−10†
oxaloacetic cyclo-aminoguanidide	16	0	ns§
normal octyl 3-methyl-phenoxyacetate	ns§	37	25
penta-chloronaphthalene*	ns§	22	14
penta-chlorophenol	ns§	0‡	− 4
penta-chlorophenoxyacetic acid*	ns§	0	ns§
penta-chlorophenyl 3-bromo-3,5-dichlorobenzoate	ns§	26	−15
penta-chlorophenyl alpha-chloroacetate	ns§	0‡	− 4
penta-chlorophenyl 2,4-dichlorophenoxyacetate	ns§	93	98
penta-chlorophenyl N-phenylcarbamate	ns§	0‡	34
phenol	5	23	− 9†
phenol 2-carboxylic-4-sulphonic acid	−14	0	−29
phenoxyacetamide	−26	0‡	ns§
phenoxyacetanilide	26	7	18†

TABLE 6—*Continued*

Compound	Test		
	A	B	C
phenoxyacetic acid	5	13	−14†
phenoxyaceto-4-bromoanilide	ns§	0‡	33
N-phenyl N′-(2-[6-aminopyridyl]) urea	ns§	16‡	ns§
phenylammonium fluoborate	0	0	31
phenyl azide	ns§	0‡	37
N⁴-phenyl biuret	ns§	0‡	ns§
N-phenylcarbamylguanidine	ns§	10‡	ns§
N-phenyl N′-carboxymethyl urea	ns§	0	ns§
N-phenyl N′-chloroacetyl urea	7	0	35†
N-phenyl-alpha-chloroacetamide	− 6	2	10†
9-phenyl-1,2-7,8-dibenzoxanthene	ns§	26	4
N-phenyl-2,4-diketo-3,4-dihydro-5,6-benzo-1,3-oxazine	ns§	0‡	2†
N-phenyldithiocarbamic acid, ammonium salt	10	7‡	− 9†
1,4-phenylene-bis-(N-alpha-naphthylcarbamate)	ns§	0	ns§
N-phenylglycine	− 8	15	12
N-phenyl N′-(2-hydroxyethyl) urea	−19	19	30†
phenyl isocyanate	−30	0	30†
phenyl isothiocyanate	ns§	0‡	20
N-phenyl N′-alpha-naphthyl urea	ns§	33‡	ns§
2-phenylphenol	20	0	.....
4-phenylphenol	−33‖	15‡	.....
2-phenylquinoline-4-carboxylic acid	1	0	−19†
N⁴-phenylsemicarbazide	ns§	0	ns§
N-phenyl thiourea	13	0‡	ns§
N-phenyl urea	ns§	5	ns§
potassium ethyl xanthogenate	−10	0	ns§
potassium methyl xanthogenate	7	0	ns§
2-isopropyl-4-chloro-5-methylphenoxyacetic acid	− 2	0	14†
normal-propyl N-(alpha-naphthyl)-carbamate	ns§	0	18
isopropyl N-(alpha-naphthyl)-carbamate	ns§	47	42
N-isopropyl O-phenylcarbamate*	−11	0	9†
normal-propyl N-phenylcarbamate	ns§	15	ns§
pyridine (sulphate)	2	0	42
pyridine-2-carboxylic acid, sodium salt	− 5	0	57†
N-2-pyridyl N′-alpha-naphthyl urea	ns§	0‡	ns§
N-2-pyridyl N′-phenyl urea	ns§	12	ns§
quinoline ethiodide	−13	0	ns§
8-quinolinoxyacetic acid*	5	29	ns§
resorcinol	25	0	0†
sodium anthraquinone-2-sulphonate	− 8	24	ns§
sodium 4-bromophenylsulphamate	5‖	0	ns§
sodium N-cyclohexylsulphamate	16	0	16†
sodium 2,4-dichlorobenzylsulphonate*	− 9	6	2†
sodium phenylsulphamate	− 9	0	ns§
sulphadiazine	3	1	ns§
sulphaguanidine	− 5	0	.....
sulphanilamide	24	17	ns§
sulphanilic acid	0	3	.....
7-sulpho-2-naphthyl N-phenylcarbamate, sodium salt	ns§	5	ns§
4-sulphophenyl N-(alpha-naphthyl)-carbamate, sodium salt	ns§	0	.....
sulphapyridine	14	28	.....
tetrabromophthalic acid, sodium salt	−18	28	ns§
N-(2-thiazolyl) N′-(alpha-naphthyl) urea	ns§	23	ns§
2-thioketo-4-phenyl-5-carbethoxy-6-methyl-1,2,3,4,-tetrahydropyrimidine	ns§	30	0
thiourea	6	33	ns§
3-tolyl isocyanate	ns§	3‡	− 5
4-tolyl N-phenylcarbamate	ns§	0	−118
N-2-tolyl urea	ns§	47	ns§

TABLE 6—*Continued*

Compound	Test		
	A	B	C
N-3-tolyl urea	ns§	0	ns§
N,N′-di-(4-tolyl) urea	ns§	0	ns§
2,4,6-tribromo-3,5-dimethylphenol	16	0	−34†
alpha, alpha, alpha-trichloroacetanilide	22	0‡	− 5†
2,4,5-trichlorobenzenesulphonyl chloride	ns§	0	22
2,2,2-trichloroethyl 2′-methyl-4′-chlorophenoxyacetate	ns§	2‡	31
alpha, alpha, alpha-trichloro-beta-hydroxy-gamma-(3-ethyl-4-pyridyl)-propane	18	18	13
4,x,y-trichloro-2-methylphenoxyacetamide	ns§	6	17
4,x,y-trichloro-2-methylphenoxyacetic acid	−17‖	4	0†
alpha, alpha, alpha-trichloro-beta-hydroxy-gamma-(4-pyridyl)-propane	8	39	ns§
2,4,6-trichlorophenol	11‖	0	25†
2,4,5-trichlorophenoxyacetamide	ns§	48	ns§
2,4,6-trichlorophenoxyacetamide	ns§	19	8
1-(2′,4′,5′-trichlorophenoxyacetamido)-anthraquinone	ns§	0	−40
2,4,5-trichlorophenoxyacetic N-(2-aminoethyl)-amide	ns§	0	114
2,4,5-trichlorophenoxyaceto-2′-bromoanilide	ns§	0‡	ns§
2,4,5-trichlorophenoxyaceto-2′-chloroanilide	ns§	.....	ns§
2,4,5-trichlorophenoxyaceto-4′-chloroanilide	ns§	0	ns§
2,4,5-trichlorophenoxyaceto-2′,5′-dichloroanilide	ns§	22	.....
2,4,5-trichlorophenoxyaceto-(diphenyl)-amide	ns§	0‡	−13
2,4,5-trichlorophenoxyacetohydrazide	ns§	10‡	ns§
2,4,5-trichlorophenoxyacetic N-(2′-hydroxyethyl)-amide	ns§	13	ns§
2,4,5-trichlorophenoxyaceto-alpha-naphthalide	ns§	0	ns§
2,4,5-trichlorophenoxyaceto-3′-nitroanilide	16	1	9†
2,4,5-trichlorophenoxyaceto-4′-xenylamide	26	0	−53†
2-(2′,4′,6′-trichlorophenoxyacetamido)-anthraquinone	ns§	0	31
4-(2′,4′,5′-trichlorophenoxyacetamido)-azobenzene	ns§	0	− 5
4-(2′,4′,6′-trichlorophenoxyacetamido)-benzene-sulphonic acid	ns§	.....	.....
2,4,6-trichlorophenoxyacetyl chloride	ns§	17	15
N-(2,4,6-trichlorophenyl)-alpha-chloroacetamide	ns§	6‡	35
N-2,4,6-trichlorophenyl N′-phenyl urea	ns§	0	ns§
2,4,6-trichlorophenylsulphamic acid (sodium salt)	12	0	24†
S-(2,4,5-trichlorophenyl)-thioglycolic acid	17	12	− 5†
S-(2,4,5-trichlorophenyl)-thioglycolic chloride	17	0‡	13†
S-(2,4,5-trichlorophenyl)-thioglycolic anilide	ns§	1‡	12
S-(2,4,5-trichlorophenyl)-thioglycolic 2′-bromoanilide	ns§	0‡	29
S-(2,4,5-trichlorophenyl)-thioglycolic 3′-bromoanilide	ns§	0‡	12
S-(2,4,5-trichlorophenyl)-thioglycolic 4′-bromoanilide	ns§	0	12
S-(2,4,5-trichlorophenyl)-thioglycolic (normal-butyl)-amide	ns§	8‡	0
S-(2,4,5-trichlorophenyl)-thioglycolic 3′-chloroanilide	ns§	0‡	6
S-(2,4,5-trichlorophenyl)-thioglycolic 2′,4′-dichloroanilide	ns§	17‡	0
S-(2,4,5-trichlorophenyl)-thioglycolic diphenylamide	ns§	0‡	8
S-(2,4,5-trichlorophenyl)-thioglycolic 3′-toluidide	ns§	12‡	0
2,4,5-trichlorothiophenol	ns§	0‡	− 8
tricresyl phosphate	10	8‡	11†
triethyl phosphate	0	8	0†
N-(3-[trifluoromethyl]-phenyl)-alpha-(2-methyl-4-chlorophenoxy)-acetamide*	ns§	23‡	37
2,3,5-triiodobenzamide	ns§	0	ns§
2,4,5-triiodobenzoic acid*	ns§	0	40
2,4,6-trinitrophenyl N-phenylcarbamate	24	11	−11
triphenyl phosphate	ns§	11	0
N-(tris-[hydroxymethyl]-methyl)-morpholine, hydrochloride	−12	0	ns§
2-xenylammonium fluoborate	−16	0	ns§
4-xenylammonium fluoborate	9	32	.....
2-xenyloxyacetamide	17¶	0	19†
2-(2′-xenyloxyacetamido)-anthraquinone	ns§	0‡	8
2-xenyloxyaceto-3-xenylamide	ns§	0‡	37

TABLE 6—*Continued*

Compound	Test		
	A	B	C
2-xenyloxyacetyl chloride	12	0	19†
N-2-xenyl N′-2-chlorophenyl urea	ns§	40‡	ns§
N-2-xenyl N′-3-chlorophenyl urea	ns§	58‡	−30
N-4-xenyl N′-2-chlorophenyl urea	ns§	0‡	7
N-4-xenyl N′-3-chlorophenyl urea	ns§	0‡	− 2
N-4-xenyldithiocarbamic acid, ammonium salt	ns§	0‡	ns§
N-2-xenyl N′-phenyl urea	ns§	0‡	45
N-4-xenyl N′-phenyl urea	ns§	0‡	ns§

A SIMPLE BIO-ASSAY METHOD FOR THE DETERMINATION OF LOW CONCENTRATIONS OF 2,4-DICHLOROPHENOXY-ACETIC ACID IN AQUEOUS SOLUTIONS[1]

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Introduction

In the course of various studies relating to the movement and persistence of 2,4-dichlorophenoxyacetic acid in different soil types and to the uptake of this growth-regulating substance from nutrient solution by the roots of broadleaf and cereal plants, the need arose for a simple bio-assay method which would accurately test for unknown concentrations of the acid in aqueous solutions. Colorimetric methods had to be discarded because no colored derivatives or degradation products could be readily formed. Various methods were tried, in which stem curvature of seedling plants and formative effects on kidney-bean and tomato plants were used as criteria; but it was soon apparent that, while these readily indicated the effects produced by low concentrations of the compound, they did not give sufficiently critical evaluations of its action.

The success attained in determining the growth-inhibitory effectiveness of certain chemicals by the corn-germination test (1) suggested that the degree of elongation of the primary root of corn seedlings might possibly be used as a criterion. In this test it was shown that low concentrations of 2,4-dichlorophenoxyacetic acid in aqueous solution had a marked inhibitory effect on root elongation of germinating corn. With certain modifications, this method met the requirements demanded of it, and the procedure and results are described below.

Material and methods

The seed material used throughout this study and for all later bio-assay determinations was a hybrid field corn, var. Silver King (Wisconsin no. 7).

The unselected kernels were first sterilized in a saturated solution of sodium

[1] Studies conducted at Camp Detrick, Frederick, Md., from September, 1944 to January, 1945, under the supervision of Dr. A. G. Norman.

hypochlorite for 3 minutes, after which they were placed in 6-inch Petri dishes on filter paper moistened with distilled water. Twenty to twenty-five kernels were placed in each dish, with the embryos facing downward. They were then allowed to germinate and grow in a dark-

TABLE 1

ROOT GROWTH, COMPUTED IN PERCENTAGE OF CONTROL, OF GERMINATING CORN SEEDS SUBJECTED TO VARIOUS CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID IN AQUEOUS SOLUTION. EACH FIGURE IS BASED ON APPROXIMATELY SEVENTY-FIVE MEASUREMENTS

Concentration (p.p.m.)	Root growth (%)	Concentration (p.p.m.)	Root growth (%)
0.001	95.8	2.5	18.5
0.005	97.0	3.0	16.0
0.01	96.0	3.0	13.9
0.01	97.0	4.0	11.2
0.1	81.6	4.0	10.6
0.1	78.2	4.0	13.2
0.2	73.0	4.5	11.2
0.2	75.1	5.0	11.0
0.3	65.0	5.0	9.2
0.4	57.3	5.0	10.5
0.4	58.0	6.0	11.5
0.5	53.1	6.0	10.8
0.5	53.0	7.0	10.0
0.5	52.6	7.0	8.8
0.75	37.8	7.5	6.4
1.0	35.3	8.0	10.1
1.0	33.7	8.0	8.9
1.0	32.1	9.0	14.2
1.5	25.8	9.0	9.4
1.5	27.2	10.0	9.8
2.0	22.0	10.0	8.9
2.0	20.5		

room at 26° C. for 48 hours. The dishes were removed from the darkroom at the end of this period, and those seeds, the primary roots of which had attained a length of 15–25 mm., were removed, measured, and placed in other 6-inch Petri dishes on filter paper moistened with 15 ml. of solutions containing various concentrations of 2,4-dichlorophenoxyacetic acid. Twelve to fifteen seeds were placed in each dish, and a record was kept of the average root lengths of the seeds (approximately seventy-five in number) used for each of the concentrations tested. After 48 hours of further growth in the darkroom, the seeds were removed, and the primary roots were again measured. By subtracting the average root length obtained at the time of the first measurement from the average root length obtained at the time of the second measurement, the amount of root elongation which occurred during the 48 hours that the seeds were in contact with the 2,4-dichlorophenoxyacetic acid solutions could be determined. The untreated series were similarly handled, with the exception that distilled water was used throughout. Solutions of unknown concentration were handled in a similar manner, with dilutions being made whenever necessary.

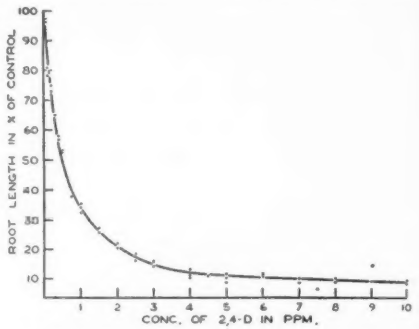

FIG. 1.—Degree of primary root elongation of germinated corn seeds when exposed for 48 hours to various concentrations of 2,4-dichlorophenoxyacetic acid (2,4–d).

Observations

The results of the numerous trials with concentrations ranging from 1/1000 p.p.m. to 10 p.p.m. are presented in table 1. The data are graphically presented in figure 1, the amount of root elongation being computed as percentage of control.

With this curve used as a basis of comparison for the determination of unknown concentrations of 2,4-dichlorophenoxyacetic acid, it is evident that the greatest accuracy will be obtained for concentrations ranging from 1/100 to 2 p.p.m. A reasonable degree of accuracy may be obtained for concentrations between 2.0 and 4.0 p.p.m.; but, as the concentrations increase, the accuracy of determination decreases. Dilutions of unknown concentrations, however, can readily be made to the more accurately tested lower levels; and repeated tests of solutions the concentrations of which were unknown to the author but known to other members of these laboratories reveal that the concentration of such solutions may be determined within the limits of a 5 per cent error.

The accuracy of the corn-germination test, especially when large samples are used, suggests that this bio-assay method may be of value in the determination of unknown concentrations of other growth-regulating substances, provided that similar reference curves are developed for the substance in question.

Summary

1. A simple method for the bio-assay of 2,4-dichlorophenoxyacetic acid in aqueous solution is described, whereby the activity of the compound is measured by its ability to inhibit the elongation of the primary root of germinating corn seed.

2. Unknown concentrations of the compound, ranging from 1/100 to 2 p.p.m., can be accurately determined by this method; it is less accurate for amounts between 2 and 4 p.p.m. and is suitable only for preliminary testing in the range of 4–10 p.p.m. and above.

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ABSORPTION AND TRANSLOCATION OF 2,4-DICHLOROPHENOXYACETIC ACID[1]

ROBERT J. WEAVER, CAPT., A.U.S., AND H. ROBERT DEROSE, CAPT., A.U.S.

Introduction

The growth-regulating substance, 2,4-dichlorophenoxyacetic acid, produces pronounced morphological responses in plants at a considerable distance from the point of application (3). Such effects have been termed telemorphic by BEAL (1). Stem curvature, epinasty, proliferation of various plant parts, and formation of gall-like growths are common responses to single droplet or spray applications of this compound. Since the acid can be detected in extremely small amounts, a study of its translocation in plants is not difficult.

HITCHCOCK and ZIMMERMAN (3) have demonstrated that synthetic growth substances can be absorbed from the soil by plants and transported upward, as are

[1] Work conducted at Camp Detrick, Frederick, Md., from June, 1944, to August, 1945, under the direction of Dr. A. G. NORMAN.

mineral salts. FERRI (2) recently studied translocation of such substances by applying the compounds to intact plants, either as solutions poured into the soil or as lanolin preparations applied around the stem. He showed that upward movement of certain regulators was intense enough to induce rooting of leaf cuttings taken from tomato and *Cleome* and planted in sand at a certain interval after treatment. He observed no movement of growth regulators on leafless *Hibiscus* cuttings when a segment of wood was removed.

The results of the experiments presented in this paper support the theory of upward movement of growth regulators in the xylem and lend evidence to the view that the phloem is the important tissue involved in downward movement.

Experimentation

ABSORPTION OF THE GROWTH REGULATOR BY PLANT TOPS

When 2,4-dichlorophenoxyacetic acid is brought into contact with aerial portions of plants, it presumably enters by penetration of the cuticle, epidermal layer, and underlying cells. An experiment was conducted which indicates that stomata may be unimportant as a portal of entry for the growth regulator. Young plants of *Nasturtium* and *Coleus* were selected for experimentation as they have stomata on only the lower surfaces of the leaves. Using a 200-ml. pressure pump, the plants were sprayed with a 0.1% solution of the ammonium salt of the acid in such a manner that in some plants only the lower surface and in others only the upper surface of the leaves was exposed to the spray. Tests were conducted on *Nasturtium* (three pots of three plants each) and on *Coleus* (two pots of one plant each). At time of harvest, 23 days after treatment, the fresh weight of leaf blades was determined, as this has been shown to be an excellent criterion in a study of growth inhibition of plants caused by this growth regulator (4). The results indicated that, regardless of the particular leaf surface sprayed, the degree of inhibition was about the same. However, when plants are exposed to volatile compounds or to an aerosol, stomata probably play a more important role.

The bark of stems appears to retard entry of the growth regulator into the transpiration stream (2). One drop of an aqueous solution of the ammonium salt of 2,4-dichlorophenoxyacetic acid was placed on cotyledons of young plants of soybean, the upper surfaces of some cotyledons being scratched with sandpaper before application. Those plants whose cotyledons were scratched were the only ones that developed curvatures, and, furthermore, they showed the least recovery. Scratching the cotyledons may have exposed some xylem tissue which may have permitted rapid entrance of the growth regulator into the plant.

Similar experiments were performed in which the compound was applied dropwise to scratched and uninjured leaves of red kidney bean. In these tests the rupturing of the leaf tissue did not aid entry of the agent into the plant. The compound entered the main portion of the plant by moving basipetally in living tissue, presumably the phloem. The cuticle and epidermal layers did not retard entrance of the compound into the leaf.

2,4-Dichlorophenoxyacetic acid often enters the leaf with great rapidity. On warm sunny days, young broadleaf plants sprayed with this compound may exhibit epinasty and stem curvature within 1 hour after treatment, indicating rapid absorption and translocation. In

another experiment droplets of an aqueous solution of the compound were placed on the base of blades of primary leaves of young soybean plants by means of a hypodermic syringe. At intervals from 1 to 24 hours after treatment the blades were snipped off just below the point of application, and growth of the plants was observed for 21 days. It was concluded that the maximum amount of the compound entered the leaf within 6 hours after treatment. A similar conclusion was drawn from experiments in which young red kidney bean plants sprayed with the compound were exposed to artificial rainfall at various intervals after spraying (5). Almost maximum damage ensued if young plants were not subjected to an artificial rainfall until 6 hours after exposure, indicating maximum entry of the compound into the plant within this period.

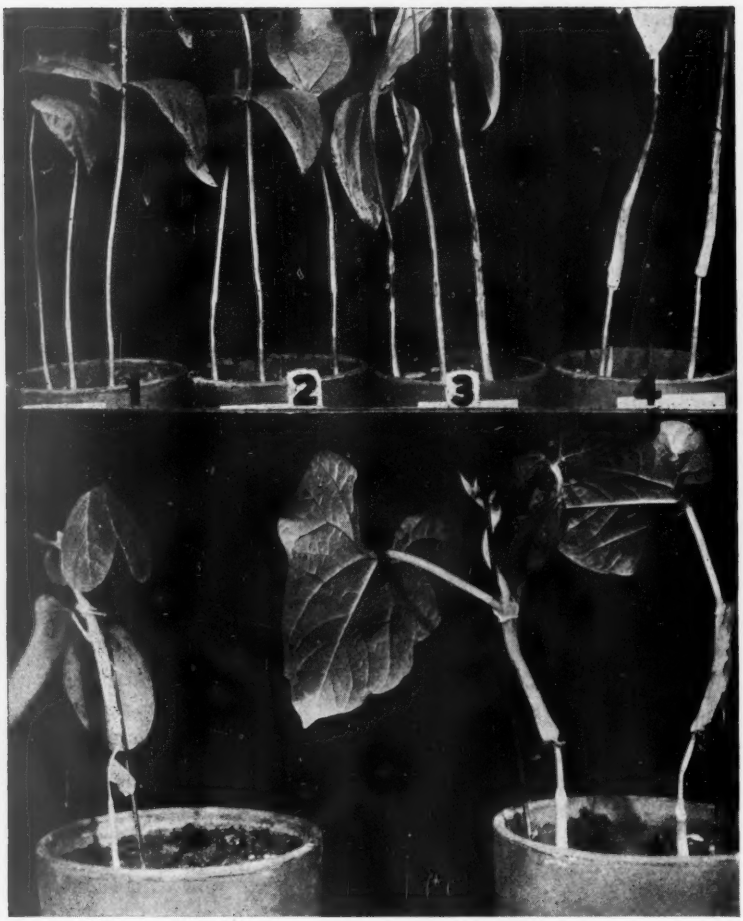
TRANSPORT THROUGH STEM SEGMENTS OF DEAD TISSUE

Soybeans at two different stages (second trifoliate leaf stage and flowering), red kidney beans at three stages (primary leaf, first trifoliate, and third trifoliate leaf), and cowpeas having three trifoliate leaves were used for experimentation. Three soybeans or cowpeas and two beans were grown in 4-inch pots. Each treatment consisted of two pots. Portions of stems about 1 inch long near the cotyledonary nodes were killed by flaming briefy with an alcohol lamp flame. After several hours the dead segments were bleached a brown or white color, and the dead portions of stems of the tender young beans and soybeans shriveled up. Otherwise the plants appeared uninjured for several days, as water uptake continued through the dead tissues of the xylem. Upward movement of the growth regulator was studied by adding to the soil 36 ml. of an aqueous solution of the ammonium salt of the acid containing 1 mg. per ml. Twenty-four hours later all plants treated with this compound showed stem curvature. The amount of curvature produced did not differ in uninjured plants and plants having a dead segment in the stem, indicating that the growth regulator readily moved upward in the transpiration stream, or xylem.

Downward movement of the stimulus produced by this compound was studied by applying a small amount of a 1% mixture in lanolin to the stem just above the dead portion. In no instance did the growth regulator move downward through the dead segment of stem (figs. 1, 2). However, this experiment does not provide unequivocal proof of lack of downward movement in the xylem of the uninjured plants, as normal functioning of the xylem was affected when stems were flamed.

TRANSPORT IN DEFOLIATED AND UNINJURED PLANTS

Red kidney beans at three stages (primary leaf, second trifoliate leaf, and flowering) and soybeans at two stages (second and third trifoliate leaf) were used. Three soybeans or two kidney beans were grown in 4-inch pots, and each test was on two pots. Some plants were defoliated at 10.30 A.M. At 3.30 P.M. on the same day both normal and defoliated plants were treated just below the second node with a small amount of a mixture of 1% of the growth regulator in lanolin. It was assumed that in uninjured plants there would be a basipetal movement of carbohydrates in the phloem, but that this flow might be much less in defoliated plants. Twenty-four hours after treatment, the bean plants in primary leaf stage showed stem



FIGS. 1, 2.—Fig. 1 (above), cowpeas 9 days after treatment with 2,4-dichlorophenoxyacetic acid. 1, control; 2, untreated but with dead stem segment; 3, compound applied to first internode of uninjured plant; 4, compound applied above dead stem segment. Note swelling of first internode and hypocotyl of 3, but that stem below dead segment in 4 is unaffected. Fig. 2 (below), soybean (left) and red kidney bean (right) several days after 2,4-dichlorophenoxyacetic acid was applied to stem above dead stem segment. Note great enlargement of stem except below dead segment.

FIGS. 3, 4.—Fig. 3 (above), red kidney beans 6 days after 2,4-dichlorophenoxyacetic acid was applied to the stem below the second node. *C*, normal and defoliated controls. Plants with leaves, 1, have greatly thickened hypocotyl and first internode, and stem curvature occurred several inches below application of the compound. Defoliated plants, 2, have no thickened stem, and angle of stem curvature is sharp and near application of the compound. Fig. 4 (below), red kidney beans 5 days after treatment. On control, 1, note one primary leaf has been largely removed by clipping. The ammonium salt of 2,4-dichlorophenoxyacetic acid applied to clipped leaf, 2, caused no growth inhibition; but the compound applied to unclipped leaf, 3, resulted in severe stunting of plants.

curvature (fig. 3). When the application was made to young uninjured plants, stem curvature occurred several inches below the point of application, but with defoliated plants there was only a sharp-angle bend in the stem adjacent to the point of application. This indicated that there was probably little or no downward movement of the compound in defoliated plants.

When final observations were made 8 days after treatment, it was evident that stem thickening and gall formation occurred below the point of application in the case of uninjured plants. However, no such responses depending on downward movement of the compound were manifested in defoliated plants (fig. 3).

Transport in plants having partially clipped leaves

Red kidney bean plants were grown in 4-inch pots, and at time of treatment they had expanded their primary leaves. Four pots were used for one test. One primary leaf of some plants was partially removed so that only about a $\frac{1}{4}$ square inch of the blade remained. The effect of such clipping, by removing actively photosynthetic leaf tissue, would be to reduce the quantity of elaborated carbohydrate material passing downward through the phloem tissues. Twelve hours after leaves were clipped, 0.02 ml. of an aqueous solution containing 10 gamma of the ammonium salt of 2,4-dichlorophenoxyacetic acid was applied to the clipped primary leaves of some plants and to the unclipped leaves of others. The liquid was applied to the bases of the blades with a hypodermic syringe. Since little plant response occurred, another 20 gamma of compound was applied in the same manner and at the same places 24 hours after the first treatment. Six hours after the second application, the stems of seven of the eight plants that received the compound on the unclipped leaf showed curvature, and the average curvature was 54 degrees. No other plants exhibited stem curvature.

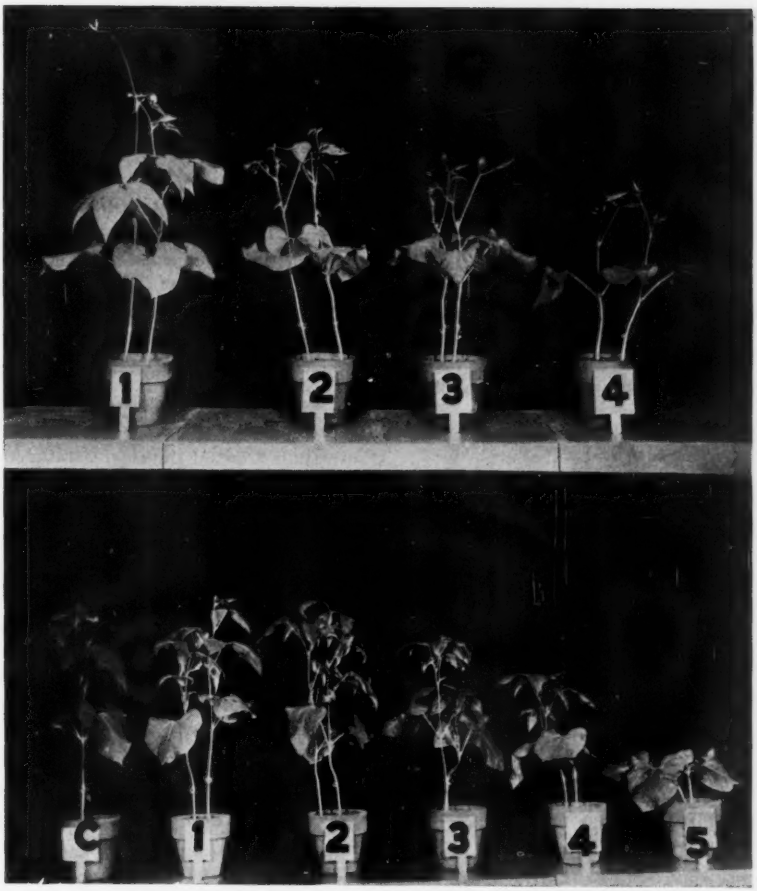
Plants receiving the compound on clipped leaves made wholly normal growth, but those receiving it on unclipped leaves were severely inhibited. Plants were harvested 12 days after treatment. The average fresh weights on a two-plant basis of trifoliate leaf blades of untreated control plants, untreated plants with clipped leaves, plants receiving treatment on the clipped leaf, and plants receiving treatment on the unclipped leaf were 5.22, 5.28, 4.96, and 1.71 gm., respectively (fig. 4). The least difference for significance between treatment means at the 1% and 5% levels was 1.17 and 0.81 gm., respectively. It is reasonable to assume that much more carbohydrate was being produced in the unclipped than in the clipped leaf, owing to the presence of greater leaf surface. Translocation of the growth regulator seems to accompany the presence of synthesized material.

Transport from various portions of primary leaves

Red kidney bean plants were grown in 4-inch pots and treated when the primary leaves were expanding. Four gamma of compound in 0.02 ml. of aqueous solution was applied with a hypodermic syringe to the base, middle, or tips of the upper surfaces of one primary leaf. Sixteen days after treatment, the fresh weights of plants above the primary node on a two-plant basis were 10.10, 6.09, 3.88, and 2.88 gm., respectively (fig. 5).

Effect of light on transport

It has frequently been observed that the curvature responses produced by the



FIGS. 5, 6.—Fig. 5 (above), red kidney beans 16 days after treatment with 4 gamma of 2,4-dichlorophenoxyacetic acid at tip (2), middle (3), and base (4) of the blade of one of the primary leaves. Control at left. All growth above primary leaves was produced after treatment. Most growth inhibition resulted when the compound was added to base of blade (4). Fig. 6 (below), red kidney beans 17 days after plants were treated with 10 gamma of the ammonium salt of 2,4-dichlorophenoxyacetic acid placed near the tip of one of the primary leaves of each plant. Control at left. The compound was removed from plants in 1, 2, 3, and 4 by removing the treated portions of the leaves 5, 8, 12, and 24 hours after treatment, respectively. Treated portions of plants in 5 were not removed. Much stunting of growth resulted when the compound remained on plant 24 hours or more.

application of 2,4-dichlorophenoxyacetic acid are less rapid and less pronounced if cloudy rather than sunny weather prevails after treatment. This phenomenon is probably related to rate and quantity of transport of carbohydrate as previously discussed. In full sun, photosynthesis may proceed actively, and both downward and upward movements in the plants would be greatly influenced by the relative conditions of light and shade prevailing.

Red kidney bean plants were grown, two per 4-inch pot, and at the time of treatment were expanding their primary leaves. At 11.00 A.M. on a sunny day some plants were placed under a small lattice shelter covered with three thicknesses of newspaper. At 8:30 A.M. on the following day, sets of plants in sunlight and in shade were treated dropwise at bases of one leaf blade with 10 gamma of the compound in 0.02 ml. aqueous solution. Since no stem curvature occurred, another 20 gamma of compound was similarly applied 3 hours later. At 9:00 A.M. on the day following treatment, the shaded plants were placed in the sun. Each treatment included four pots.

TABLE 1

AVERAGE CURVATURE (IN DEGREES) OF STEMS OF PLANTS GROWING IN SUNLIGHT AND SHADE

	TIME INTERVAL AFTER TREATMENT		
	4 hours	24 hours	28 hours
Shade	0	4	9
Sunlight	15	30	36

The amount of stem curvature was measured with a protractor. Data in table 1 indicate that most stem curvature occurred in plants growing in the light. Twenty-four hours after treatment, all treated plants in light showed curvature.

At the time of harvest, 12 days after treatment, controls had three trifoliate leaves. Average fresh weights of trifoliate leaf blades on a two-plant basis for controls in sunlight, controls in shade, treated plants in sunlight, and treated plants subjected to shade were 5.66, 5.82, 1.94, and 3.23 gm., respectively. The least differences for significance of treated means at 5 and 1% were 0.54 and 0.78 gm., respectively. Treated plants in shade produced significantly less weight of trifoliate leaf blades than controls, and treated plants in sunlight produced significantly less than treated plants in shade.

An interesting observation was that the treated leaves of plants in the dark curved upward so that the leaf tip was often over the pulvinus. Under conditions of darkness food materials are often transferred in an upward direction from the cotyledons to the leaves. The fact that the growth regulator seems to move readily in the direction of movement of elaborated food materials perhaps explains the recurving of the leaf.

This experiment indicates that movement of the compound is either more rapid or occurs in greater amount, or both, when rapid movement of synthesized carbohydrate occurs. There is indication that more ultimate injury is done when there is rapid translocation of the compound.

SPEED OF TRANSLOCATION

It has been shown that the speed of upward movement of growth substance is proportional to the transpiration rate of the plant (3). This supports the theory that solutes rise in the transpiration stream of the xylem. The velocity of movement of certain growth regulators

is influenced by the photosynthetic activity of the plant, age of the tissue involved, and several other factors.

An experiment was performed to determine the speed of transport of the growth regulator in the primary leaf of the kidney bean. Plants were grown two per 4-inch pot and treated when the primary leaves were expanding. Each treatment included three pots. Aqueous drops of 0.04 ml. containing 40 gamma of the ammonium salt of the acid were placed on the upper surface of the primary leaf near the center of the leaf. The plants were treated at 9:00 A.M. on a clear sunny day. At intervals of 1 to 72 hours after application, the leaves were snipped close to the plant stem. The distance from the center of the leaf to the base of the petiole was about $1\frac{3}{4}$ inches. Ten days after treatment the plants were harvested and average fresh weights of trifoliate leaf blades obtained. Figure 7 indicates that treated leaves had to remain attached to the plants for over a 24-hour period for attainment of maximum growth inhibition. The amount of such inhibition is proportional to the quantity of growth regulator transported from the treated leaf into the plant stem. Leaves of untreated plants were snipped off at several intervals after treatment, but this had little effect on subsequent growth.

A similar experiment was performed in which drops containing 10 gamma of the compound were placed on the primary leaf of kidney beans toward the tip of the leaf. At intervals of from $\frac{1}{2}$ to 24 hours after application, the leaves were snipped at one-third the distance from the base, thereby removing the treated area. In general, the results of this experiment were similar to those described in the preceding paragraph (fig. 6).

Regeneration studies were performed to determine whether prompt cutting back or clipping of a crop after spraying with a growth regulator would prevent or reduce the extent of the injury that might otherwise be caused. The results would be dependent on the speed at which the substance is transported from the upper to the lower plant parts. Alfalfa was grown in 6-inch pots, and at time of treatment the plants had an average foliage height of 12 inches. Each treatment included two pots. Plants were sprayed with an aqueous solution of the ammonium salt of the acid at a rate of 100 mg. in 10 ml. per square yard of ground surface. Plants were then clipped at a height of 2 inches at intervals of 1, 6, 13, 24, and 48 hours after treatment. Unsprayed controls were clipped, and one set of sprayed plants was not clipped. At time of harvest, 27 days later, the sprayed unclipped plants were almost dead, and the unsprayed clipped plants had made much new growth (fig. 8). Regeneration of plants clipped 1 hour after treatment was considerable, although the

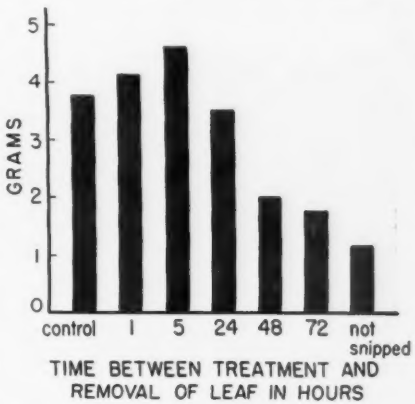


FIG. 7.—Average fresh weights on a two-plant basis of trifoliate leaf blades of red kidney bean, the primary leaves of which were treated with the ammonium salt of 2,4-dichlorophenoxyacetic acid and then removed after various intervals.

green weight was less than half that of the clipped but unsprayed control. Only a few stems of plants clipped 6 hours after spraying sent up new growth, and almost no growth occurred if the interval between spraying and clipping was 13 hours or more. At harvest all plants were clipped at a height of 2 inches and the fresh weights on a two-pot basis determined. The weights for untreated controls, for unsprayed plants clipped at the beginning of the experiment, and for plants sprayed and clipped 1, 6, 13, 24, and 48 hours after treatment were 38.1, 22.5, 10.8, 3.4, 2.0, 00, and 00 gm., respectively. The weight of plants treated but not clipped was 10.4 gm. The results indicate that there is a rapid downward transport of the growth regulator in alfalfa.

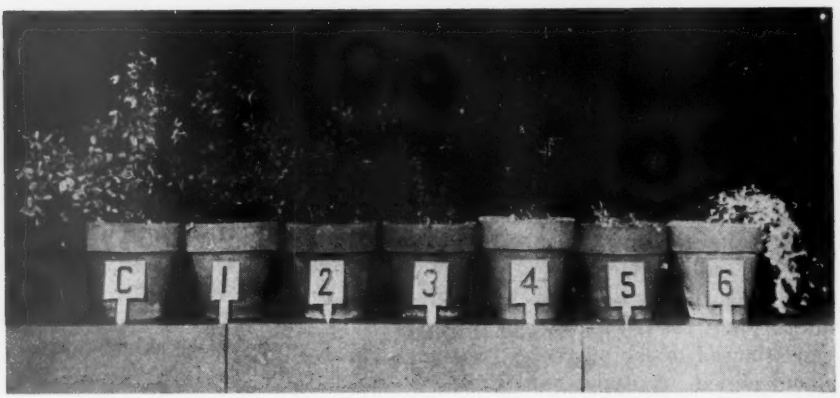
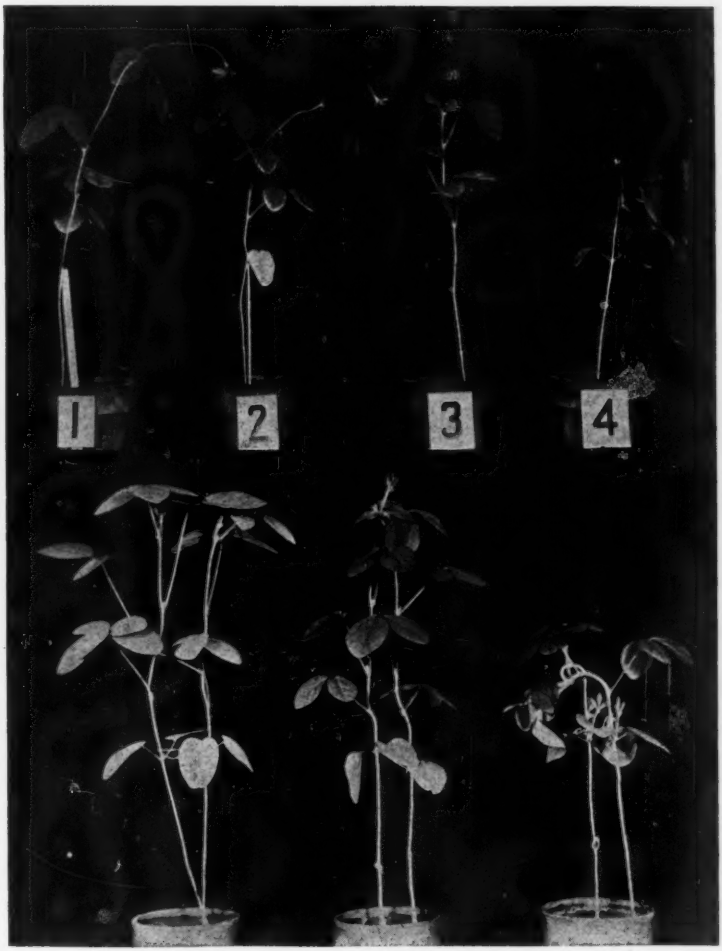


FIG. 8.—Alfalfa 27 days after being sprayed with ammonium salt of 2,4-dichlorophenoxyacetic acid. *C*, control; 1, control, clipped; 2, treated, clipped after 1 hour; 3, treated, clipped after 6 hours; 4, treated, clipped after 13 hours; 5, treated, clipped after 24 hours; 6, treated, not clipped.

INFLUENCE OF AGE OF TISSUES ON ABSORPTION AND TRANSLOCATION

An experiment was performed in which equal applications of 2,4-dichlorophenoxyacetic acid were made to successive leaves on young plants of soybean, tomato, and cotton. Beginning with the lowest leaves, aqueous droplets containing 5 or 25 gamma of compound were placed on the first, second, and third leaves of replicate plants. Sixteen days after treatment, untreated soybean plants, plants receiving compound on the first leaf, on the second leaf, and on the third leaf had heights of 60, 60, 40, and 30 cm., respectively (fig. 9); for tomatoes the heights were 32, 35, 27, and 27 cm. These results indicate that the younger the leaf the greater the ultimate inhibition of growth of the plants, when plant stature is used as a measure of growth. There is as yet no satisfactory explanation of this observation. Proximity to the apical meristem, ease of entrance into or of absorption by the leaf, rapidity or readiness of transport of the compound or its effects, all may be involved.

In a second experiment which yielded similar results 100 gamma of the compound were applied to the upper or second trifoliate leaf of soybeans, or to the lower or primary leaf. Seventeen days after treatment, plants, the upper leaves of



FIGS. 9, 10.—Fig. 9 (above), soybeans received 25 gamma of 2,4-dichlorophenoxyacetic acid as a droplet on primary leaf, 2, second leaf, 3, and third leaf, 4. Control is at left. Treatment of younger leaves caused more stunting of growth. Fig. 10 (below), soybeans 17 days after 100 gamma of 2,4-dichlorophenoxyacetic acid was added to primary leaves (middle), and second trifoliate leaves (right). Control at left. Note that when second trifoliate leaf was treated, plant was stunted and produced axillary shoot. Plants whose primary leaves were treated had enlarged hypocotyls and first internodes.

which were treated, showed severe stunting (fig. 10). Stems were bent, and no flowers had formed. Axillary shoots 1–5 inches long had grown from the cotyledonary or the second node. Leaves had a deep green color, and gall-like calluses had formed along veins of treated leaves, especially near the basal portions. Plants whose lower leaves had been treated were flowering and appeared normal, except that young leaves were deformed. The evidenced by gall formation and stem thickening. When the hypocotyl of a young kidney bean plant was treated, the swelling of the stem was usually limited to the hypocotyl, but treatment of the first internode produced stem thickening both in hypocotyl and in first internode (fig. 11). Leaves which expanded after plants were treated showed typical abnormalities induced by the agent and were evidence of subsequent upward

FIG. 11.—2,4-dichlorophenoxyacetic acid applied to first internode of red kidney bean (left) caused both first internode and hypocotyl to enlarge. Treatment of hypocotyl (right) had little effect on first internode.

hypocotyl and first internode showed much more swelling than the corresponding parts of the other plants. Average fresh weights of leaf blades for plants whose upper and lower leaves were treated on a two-plant basis were 3.75 and 4.46 gm., respectively.

Other experiments were carried out in which applications of the compound in lanolin were made to various portions of stems of kidney bean, tomato, cowpea, and soybean, and the subsequent effects, inhibitory and formative, noted. In the kidney bean plants the movement of the agent seemed to be chiefly downward, as movement of the compound. In general, when lower portions of stems were treated, the compound seemed to move downward and a minimum over-all stunting of plant growth occurred. When upper portions of stems were treated, apical growth was inhibited, much over-all stunting of plants occurred, and axillary shoots often developed.

Summary

1. The pronounced morphological response in plants to 2,4-dichlorophenoxyacetic acid is useful in studies of translocation.

2. The regulator passed upward but not downward through dead segments of stems.

3. Stomata did not appear to be important portals for entry of the compound into the leaves when it was used in aqueous sprays. Leaves of young soybeans absorbed maximum amounts of the growth regulator within 6 hours after application.

4. The movement of food materials was regulated by partial clipping of leaves, defoliation, and reduced light intensity. When the growth regulator was applied to aerial portions of plants, the substance readily moved downward whenever translocation of synthesized materials occurred.

5. More extensive growth inhibition of young red kidney bean plants at primary leaf stage resulted when the substance was added to the base of the blade than when added near the tip. When the compound was added to a primary leaf of a young bean plant more extensive growth inhibition resulted when translocation of the growth substance was rapid than when it was slow.

6. When the growth regulator was added to the primary leaf of kidney beans, it was rapidly translocated basipetally to the stem. In one experiment leaves treated with 40 gamma of the growth regulator had to remain on the plant for 24 hours for maximum amount of compound to reach the stem. Experiments in which alfalfa was sprayed with the growth regulator and then clipped at subsequent intervals showed that much of the compound was translocated from upper to lower plant parts within 1 hour. The velocity of downward movement is markedly influenced by the photosynthetic activity of the plant and the age of the tissue involved.

7. When upper portions of stems or of younger leaves were treated with the compound, apical growth was inhibited, much over-all stunting of plants occurred, and axillary shoots often developed. When lower portions of stems or of older leaves were treated much less stunting of plants occurred. There is as yet no explanation for this occurrence. Proximity to the apical meristem, ease of entrance into the leaf, rapidity or readiness of transport of the compound—all may be involved.

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HISTOLOGICAL RESPONSES OF THE KIDNEY BEAN TO AQUEOUS SPRAYS OF 2,4-DICHLOROPHENOXYACETIC ACID[1]

CARL P. SWANSON, LT., U.S.N.R.

Introduction

Recent interest in the herbicidal activity of phenoxyacetic acids and in their physiological similarity to other growth-regulating substances suggested that a histological study of the responses produced might throw some light on the manner by which these compounds affect various plant tissues. The compound 2,4-dichlorophenoxyacetic acid was selected for study because of its effectiveness at very low concentrations and because, unlike certain other growth-regulating substances studied, its action is systemic rather than local in nature.

Material and methods

The kidney beans used in this study had their primary leaves fully expanded at the time of treatment, and the second internodes had attained lengths of 5–10 cm. The 2,4-dichlorophenoxyacetic acid was applied in the form of a 0.1% aqueous spray with an atomizer at a rate of 30 ml. per square yard (a rather heavy rate of application). This constitutes a lethal dose for kidney-bean plants at this stage of development, but the plants survived sufficiently long to yield satisfactory material for histological study. Following treatment, the plants were grown under normal greenhouse conditions, and collection of material for fixation was made at regular intervals. The collected material was fixed in a formalin-acetic acid-alcohol solution and passed through the butyl alcohol series into paraffin. All sections were cut at 12μ and stained according to the safranin-fast green schedule.

Gross responses

The responses of the young kidney bean to 2,4-dichlorophenoxyacetic acid are striking. Within an hour or two after the spray treatment, a sharp bending of the first internode takes place (this response is most rapid in bright sunlight). The second internode may likewise bend or twist, and a severe epinasty usually follows. It is evident that a differential elongation of cells must take place to produce these curvatures. The plants have the appearance of being severely wilted, but in no instance was there an indication of wilt, the stems and foliage being noticeably more turgid than untreated material. The plant, as a rule, does not later regain an upright position but retains its curvature permanently.

Within 24 hours a yellowing of the younger portions of the stem occurs, particularly along the rigid portions of the second internodes. This yellowing, due presumably to a loss of chlorophyll and, to some extent, of plastids, increases in extent with the passage of time. The primary leaves, however, remain green and may even take on a darker hue. After 48 hours a noticeable thickening of the second internode becomes evident. This thickening may take various forms. If the second internode is 5 cm. or less, the entire region swells evenly and finally becomes an elongated gall, through which protuberances of various shapes and sizes emerge. Longer internodes, on the other hand, are more likely to form

[1] Studies conducted at Camp Detrick, Frederick, Md., from September, 1944, to September, 1945, under the supervision of Dr. A. G. Norman.

localized galls at the tip and base. The axillary buds, too, enlarge in size and develop into galls tipped by tiny leaves, which do not increase in size.

The first internode and the hypocotyl are slower to respond, but each takes on a characteristic appearance. The internode, which bends just below the primary leaf node, becomes distinctly ridged, almost corrugated; increases gradually in girth throughout its entire length; and then bursts its cortex as the internally formed root primordia push outward. The hypocotyl, which may or may not increase in diameter, reacts by forming four vertical rows of root primordia, giving the stem a somewhat squared appearance in cross-section. These rows of root initials, when followed downward, merge with the tetrarch root system underground.

The formation of roots in considerable profusion on the stem, petioles, and even the midribs of leaves is one of the most characteristic of the responses. Generally, root primordia are formed only on the stem; but, where heavy local applications of 2,4-dichlorophenoxyacetic acid are made on the leaves, roots may appear on the petioles and midribs. When moist conditions exist in the greenhouse, as when the potted plants are closely packed together, the emerging root primordia continue to elongate, giving the plants a distinctly hairy appearance. At the base of the hypocotyl, the roots become fasciated, extending outward as wedges of tissue at right angles to the stem axis.

Following such a heavy application of 2,4-dichlorophenoxyacetic acid, the stoppage of growth is complete except as it involves an internal cell proliferation and differentiation. The young trifoliate leaves do not expand, and they are usually incorporated into the terminal gall, with only the tips of the young leaves extending beyond the end of the gall.

Histological responses

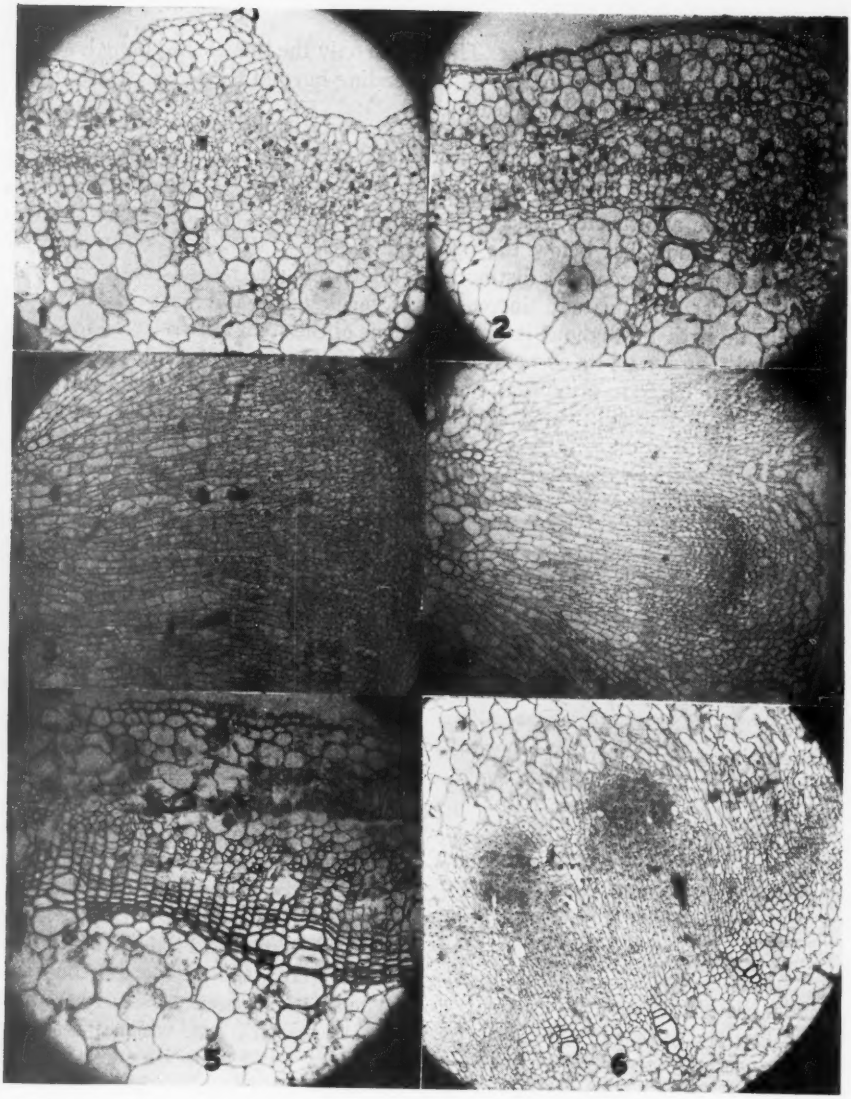
In order to examine adequately the histological effects produced by 2,4-dichlorophenoxyacetic acid on the various tissues of the kidney-bean stem and to determine the responsiveness of tissues of varying ages and maturity, three portions of the stem were selected for study, namely, those portions of the hypocotyl, first internode, and second internode about 5–10 mm. below the nodes. DOUTT (3) had previously determined that the hypocotylar organization immediately below the cotyledons was essentially that of subsequent internodes. The petiole of the primary leaf was briefly studied.

SECOND INTERNODE

The second internode, being relatively immature, responded to a greater degree than did the older tissues of the first internode and the still older tissues of the hypocotyl. The responses will be described in some detail.

At the time of treatment the second internode consisted largely of immature cells (fig. 1). The pith was solid. The protoxylem, radially arranged, was not heavily lignified, and the protoplasts and nuclei were still visible in the outer vessels. Other primary tissues were readily identifiable; but, while a fascicular cambium was evident in some sections, no secondary tissue had been laid down. The ray cells, extending through the primary phloem, showed no evidence of any radial elongation. The pericyclic fibers, two to five cells deep over the vascular bundles, were well differentiated, but their walls were still thin, and their contents had not begun to disintegrate.

In longitudinal section the cortical and



FIGS. 1–6.—Fig. 1, transverse section of the second internode of an untreated kidney bean. Fig. 2, transverse section of second internode of kidney bean, 36 hours after treatment. Interfascicular endodermis, cambium, and ray cells show activity. Fig. 3, transverse section of second internode, 72 hours after treatment. Only primary xylem remains unaffected; other tissues probably embryonic at time of treatment. Fig. 4, second internode, 96 hours after treatment. Root primordia well developed at this stage. Fig. 5, transverse section of first internode of untreated kidney bean. Fig. 6, first internode, 90 hours after treatment. Relationship of root primordia to ray areas clearly shown. Note radial elongation of endodermis and the formation of short, pitted tracheids.

cambial cells were considerably elongated; and the endodermal, parenchymatous, and ray cells were roughly twice as long as they were wide. No marked activity was noticed in any of the tissues, although the meristematic appearance of phloem parenchyma and ray cells indicated that they were still capable of further division.

The first indication of histological activity was evident 9 hours after the application of 2,4-dichlorophenoxyacetic acid. The cytoplasm and nuclei took on a much deeper stain, and the nuclei were enlarged considerably in the endodermis, phloem parenchyma, cambium, xylem parenchyma, and ray cells. A somewhat less marked increase in staining intensity characterized the cells of the pith and the cortex. No perceptible increase in cell size was noted in any of the tissues. No measurements of the longitudinal axes of cells were made to determine which tissue was responsible for the distinct curvature of the stem. Division figures up to metaphase were observed in the phloem parenchyma and ray cells, but the fact that no further division figures were found at 24 hours suggests that these tissues were still sufficiently meristematic to be dividing normally. It appears likely that the application of 2,4-dichlorophenoxyacetic acid hastened the division cycle of those cells which were already undergoing mitosis but that it did not stimulate others to divide until later.

At the end of 24 hours the endodermis had visibly enlarged in a radial direction, and the endodermal nuclei were undergoing enlargement prior to entering early prophase.

Between 36 and 48 hours the endodermis had divided both transversely and tangentially, with the greater number of divisions in the transverse plane (fig. 2). The endodermal cells over the vascular bundles were considerably slower to react than those over the interfascicular regions, dividing some 12–18 hours after the others. The fascicular cambium was equally active at this time; tangential division occurred rapidly in the vicinity of the outer primary vessels. The ray cells and the phloem parenchyma were somewhat slower to respond, but mitotic activity had been initiated. Cortical cells adjacent to the endodermis were beginning to enter prophase, but no activity other than an intense nuclear staining was to be found in the pith and xylem parenchyma.

By 72 hours a considerable mass of proliferated tissue had been produced, particularly by the developing cambium. This tissue, together with the phloem parenchyma, gave rise to a broad zone of rapidly dividing cells, which, as they increased in number and elongated in a radial direction, pushed the primary tissues farther and farther apart (fig. 3). This illustration is from a somewhat younger section of the second internode than those shown in figures 1 and 2, but it is more representative of the reaction of this portion of the stem to 2,4-dichlorophenoxyacetic acid. The entire region between the xylem and the cortex became a mass of proliferating cells, and the identity of the individual tissues, with the exception of the rays, was lost. The ray derivatives may be readily identified by their considerable radial length.

In figure 3 it will be noticed that the pericyclic fibers and the sieve tubes are not distinguishable. It is probable that they were sufficiently immature to respond to the application of 2,4-dichlorophenoxyacetic acid and thus to merge with other proliferating cells; but in other sections of the second internode, where some differentiation had taken place, these cells did not divide.

No typical phloem or xylem cells were laid down during this period, although short, tracheid-like cells with simple pits were scattered haphazardly throughout the proliferated zone. This zone, at 72 hours, may be thirty to forty cells in depth, as contrasted to the five- to seven-cell thickness of the phloem-cambium region of a normal mature stem. A stem of similar age would still possess a relatively immature secondary xylem and phloem, but it would be clearly recognizable. The 2,4-dichlorophenoxyacetic acid, then, not only stimulates the meristematic tissues into a very rapid division period but, by maintaining meristematic activity in all the cell derivatives, also prevents the maturation of any of the tissues which are normally laid down.

When a stem with the primary tissues fully developed was treated with 2,4-dichlorophenoxyacetic acid, the results were somewht different (cf. figs. 3 and 4). In figure 4, which is a cross-section of an older section of the second internode, the small groups of sieve tubes may be seen over the vascular bundles, with the pericyclic fibers pushed aside by the proliferating ray tissue. The root primordia, which will be discussed in more detail below, arose in the interfascicular regions from the cells of the rays and pushed outward, disrupting the cortex and the epidermis. These root primordia were initiated almost as soon as the ray cells underwent proliferation, but it was not until 72–84 hours after treatment that they could be identified as such with certainty.

Later stages, up to 120 hours, were studied; but, although cell division continued and the root primordia emerged through the cortex, the details added nothing to those already described for earlier stages.

First internode

The histological study of the first internode was confined to that region just below the primary leaves where the leaf traces had not yet begun to pass outward. This was the portion of the internode which responded initially to 2,4-dichlorophenoxyacetic acid by bending sharply. Figure 5 shows a section of a first internode with the secondary xylem well developed, a condition somewhat more advanced in differentiation than was usual at this stage of development. In most instances the secondary xylem was but one or two cells in thickness.

The pattern of change in the first internode was similar to that in the less mature second internode. The changes, however, were less dramatic. At 24 hours a deeper staining of the endodermis, ray, cambium, and phloem parenchyma was noted. At 48 hours the ray cells and interfascicular endodermis had divided, while the cambium, phloem parenchyma, and fascicular endodermis were in various prophase stages of division. Frequently an entire ray area, from pith to cortex, showed activity. Binucleate cells were often found, indicating that nuclear division progressed at a more rapid rate than did cell division. The 2,4-dichlorophenoxyacetic acid, however, caused no upset in the spindle mechanism or in chromosome-spindle co-ordination. All division figures were monotonously regular.

At 72 hours the cambium and its derivatives, which remain meristematic, had formed a zone of proliferating cells, with a greater degree of proliferation over the interfascicular regions. At 90 hours root primordia were clearly evident (fig. 6), although these had had their initiation somewhat earlier. As nearly as could be determined, it was the

ray cells immediately under the cortex which initiated the formation of root primordia. As the root primordia developed, the core appeared to be derived from the ray cells, as was the region of the histogen, while the flanking regions were made up of derivatives from the cambium and phloem parenchyma. The apex of the root, composed of many small cells, continued to proliferate at a rapid rate, passing through the cortex and epidermis to emerge as a small protuberance on the surface of a swollen stem. The core of the root was generally composed of somewhat elongated cells, with simple-pitted tracheids forming in a scattered fashion along the boundary between the core and the flanking zone. It could not be determined whether the tracheids arose from cells of the ray, the cambium, or the phloem or from all three sources.

Endodermal cells capping the root primordia were disrupted as the root passed outward, and the groups of sieve tubes and pericyclic fibers were pushed to one side. The endodermal cells, however, had undergone a considerable radial elongation before disruption (fig. 6). This was particularly true of those cells which lie over the fascicular regions and which flank the root primordia.

Some differentiation of xylary elements occurred following treatment with 2,4-dichlorophenoxyacetic acid. The cells laid down were not the usual elongated tracheal cells characteristic of the kidney bean but were short, with simple pits, and not very heavily lignified. Similar cells were differentiated in the proliferated zone derived from the cambium (fig. 7). They may form islands of tracheids or may exist singly, and they were more generally formed in the areas between root primordia than at the bases of such roots.

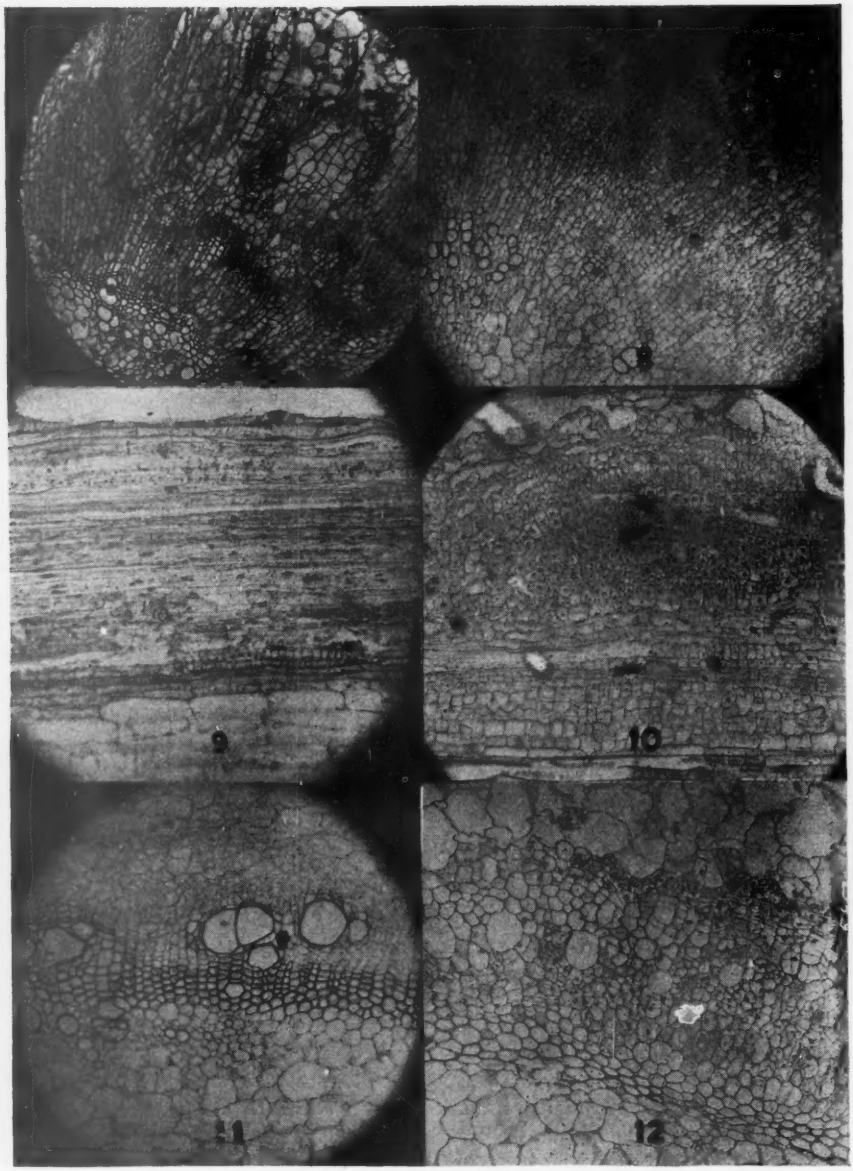
The pith exhibited no response. The xylem parenchyma was only slightly responsive, but occasionally areas were found in which the primary xylem elements were forced apart by the proliferating xylem parenchyma (fig. 8).

Figures 9 and 10 reveal the longitudinal aspects of change which take place in the first internode. In the untreated section (fig. 9) the considerable length of all tissues, with the exception of the endodermis, cortex, and epidermis, is evident. The cambium has not yet laid down any secondary tissues, although it is itself well differentiated. Figure 10, taken in the region of a root primordium, reveals quite clearly some of the changes brought about in response to 2,4-dichlorophenoxyacetic acid. The cambium no longer consists of fusiform elements, since many transverse divisions reduced the length of the cells. Its derivatives matured as short tracheids. The sieve tubes and the pericyclic fibers were unchanged, but the phloem parenchyma and the ray cells divided many times to form the root primordium, which, as it passed outward, disrupted the endodermis, cortex, and epidermis.

Hypocotyl

The hypocotyl responded to 2,4-dichlorophenoxyacetic acid in much the same manner as the first internode, except that the tempo of change was slower.

Figure 11 is a cross-section of an untreated stem. Figure 12 shows the changes which took place 72 hours after treatment. The endodermis appeared to divide more frequently in the hypocotyl than in other sections of the stem, but the other changes were similar, except that the pattern of root formation was somewhat different. In the first internode there were fifteen to twenty root primordia, arranged in a spokelike fashion

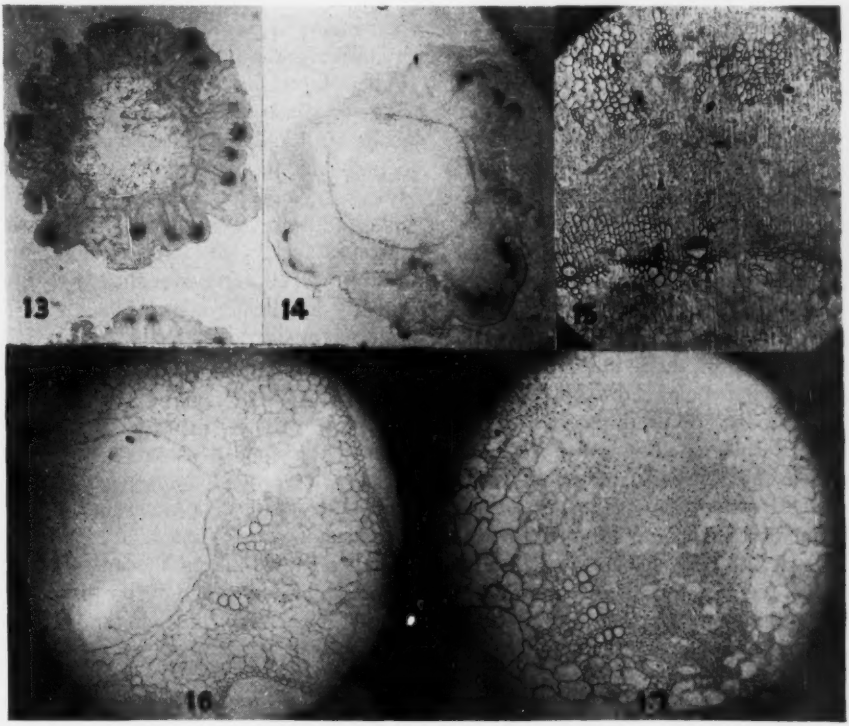


FIGS. 7–12.—Fig. 7, first internode, 120 hours after treatment. Note groups of xylary elements differentiated in the proliferated zone. Fig. 8, first internode, 96 hours after treatment. Proliferation of xylem parenchyma has disorganized the arrangement of elements in the vascular bundle. Fig. 9, longitudinal section of first internode of an untreated bean plant. Fig. 10, longitudinal section of first internode, 84 hours after treatment. FIG. 11, transverse section of the hypocotyl of an untreated kidney bean. Fig. 12, transverse section of hypocotyl, 72 hours after treatment.

(fig. 13). In the hypocotyl a distinct tetrarch arrangement was usually found (fig. 14). This is undoubtedly a reflection of the tetrarch system, which characterizes the bean root. Between the root primordia there was a much greater production of tracheal elements in the proliferated zone than was found in the younger portions of the stem, and it was considerably more in evidence at the base of the hypocotyl than at the top (fig. 15).

Petiole of the primary leaf

The sequence of histological events of this portion of the plant has not been investigated so thoroughly as have the stem portions previously described, but a comparison of figures 16 and 17 will reveal the changes that have taken place. Figure 17 shows that the phloem-cambium region had undergone a considerable enlargement due to the rapid proliferation of cells. This expansion displaced the pericyclic fibers, and the di-



Figs. 13–17.—Fig. 13, first internode, 120 hours after treatment, showing the numerous root primordia which form between the vascular bundles. Fig. 14, hypocotyl, 120 hours after treatment, showing the tetrarch arrangement of the root primordia (one group of primordia has been lost). Fig. 15, hypocotyl, 120 hours after treatment, showing the differentiation of tracheid-like elements in the proliferated zone. Fig. 16, transverse section of an untreated petiole. Fig. 17, petiole, 96 hours after treatment, showing proliferation occurring in the phloem-cambium region.

viding cells invaded the fundamental parenchyma surrounding the bundles. There had been some stimulation of the fundamental parenchyma over and between the bundles, but, in general, this tissue did not show any marked responsiveness. The xylem parenchyma was slightly responsive. No xylary elements appeared to be differentiated in the petiole following the treatment.

Discussion

It has been adequately shown (1, 2, 4, 8, 10, 11, 12, 13) that 2,4-dichlorophenoxyacetic acid and other substituted phenoxyacetic acids differ from other growth-regulating substances in their influence on formative disturbances when used in extreme dilutions and in the systemic nature of their effects. The combined effect of these influences on the physiological balance within the plant undoubtedly determines the unique properties which characterize 2,4-dichlorophenoxyacetic acid as a herbicidal agent. In other respects the physiological action of this compound bears a close similarity to that of the more localized growth-regulating substances, in that the formative disturbances are somewhat of the same nature as regards tissue responses and cell proliferation. However, as MULLISON (9) indicates in a table comparing the histological responses of growth-regulating substances tested on the kidney bean, each of the substances tested to date produces a sufficiently characteristic pattern of events as to suggest that each sets in motion, interrupts, or alters a pattern of physiological mechanisms peculiar to itself. The 2,4-dichlorophenoxyacetic acid, in turn, shares this individuality of effect, although as yet no data from this study, or from other investigations, yield any clue as to the mechanisms involved.

The 2,4-dichlorophenoxyacetic acid produces histological responses very similar to those induced by indoleacetic acid (6) and l-tryptophane (5). The endodermal, phloem, ray, and cambial cells are greatly activated by all three; but 2,4-dichlorophenoxyacetic acid does not stimulate the formation of vascular bundles in the endodermis and phloem, being similar in this respect to tetrahydrofurfural butyrate (9). Alpha-naphthalene acetamide, on the other hand, has very little effect on the phloem (7), while 2,4-dichlorophenoxyacetic acid is equally ineffective in stimulating the xylem, xylem parenchyma, and pith. Like indoleacetic acid and alpha-naphthalene acetamide but unlike tetrahydrofurfural butyrate and l-tryptophane, 2,4-dichlorophenoxyacetic acid induces considerable root formation. It is evident, therefore, that, while similarities of response exist, differences of response are equally obvious.

From the point of view of herbicidal effectiveness, 2,4-dichlorophenoxyacetic acid and others of a somewhat similar chemical structure rank high among the so-called "weed-killers" because of their severe inhibitory action on broadleaf plants at extreme dilutions and at low rates of application (2, 4, 10, 11, 12, 13). Unpublished observations on a number of the substituted phenoxyacetic acids and their derivatives tend to suggest that the greater the degree of cell proliferation and formative disturbance induced by a substance, the more effective is that substance as a herbicidal agent. Histological studies, therefore, provide an excellent criterion of effect when comparative observations are being made on the herbicidal qualities of various growth-regulating substances; but they must be supplemented by other studies, since the selective herbicidal action of these substances on various plant species is a recognized fact (2, 10, 11).

Summary

1. Aqueous-spray applications of a 0.1% solution of 2,4-dichlorophenoxyacetic acid induce a considerable degree of cell proliferation in the stem of the kidney-bean plant.

2. The histological changes induced are as follows:

Epidermis.—No response.

Cortex.—Little response, although cells adjacent to the endodermis frequently enlarge and divide.

Endodermis.—Active; transverse and tangential divisions occur, as well as considerable radial elongation.

Pericyclic fibers.—Active if embryonic, but show no response if differentiated.

Phloem.—Sieve tubes inactive; phloem parenchyma very active and may enter into root formation or differentiate into isolated tracheal groups.

Cambium.—Very active; derivatives remain meristematic, no phloem or xylem being regularly differentiated, although tracheal groups may be laid down; takes part in root formation.

Rays.—Very active, being the primary tissue involved in root formation.

Xylem.—Formation of xylary elements greatly inhibited and, when occurring, much disorganized; xylem parenchyma slightly active, but to no great extent.

Pith.—No response.

3. In general, it may be stated that meristematic tissues and those capable of reverting to a meristematic condition are most readily affected by 2,4-dichlorophenoxyacetic acid. The derivatives of such tissues remain meristematic for considerable periods, and, if differentiation occurs, it is never in an orderly fashion.

4. The effect of 2,4-dichlorophenoxyacetic acid is systemic in nature, even at relatively low concentrations. In this respect it differs from other growth-regulating substances.

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EFFECT OF SPRAY APPLICATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID ON SUBSEQUENT GROWTH OF VARIOUS PARTS OF RED KIDNEY BEAN AND SOYBEAN PLANTS[1]

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Introduction

Small quantities of 2,4-dichlorophenoxyacetic acid produce marked responses in plants when applied in amounts less than lethal. Some of the responses produced in the red kidney bean plant have been described by SWANSON (2). The various plant parts are affected to different degrees. The present investigations were concerned with growth and weight changes produced by this compound in various parts of kidney bean and soybean plants and had as their prime objective the determination of the best and simplest criterion that could be used experimentally as an index of overall growth-inhibitory effect in the evaluation of the herbicidal activity of growth-regulating substances.

Experimentation

EXPERIMENT I: RED KIDNEY BEAN

Red kidney beans were planted in 4-inch pots, and before treatment were thinned to two plants of uniform size per pot. The plants were grown under ordinary greenhouse conditions and were treated 14 days after planting, when the first trifoliate leaves were expanding.

Three aqueous solutions of the growth regulator were prepared, each of which contained 0.5% Carbowax 1500. The plants were sprayed in 50-ml. amounts at rates of 0.001, 0.01, and 0.1 gm. per square yard of plot area with 200-ml. pressure-sprayers under 100 lb. pressure. Plants treated with the lowest amount (0.001 gm. per sq. yd.) appeared only slightly affected. Curvature of shoots and leaf epinasty occurred soon after treatment. Subsequent growth seemed little retarded, although formation of leaves with abnormal veination occurred. Plants treated with the highest concentration were severely inhibited. Shoot curvature and epinasty were soon manifested, and further growth was much inhibited. Thickening of stems and petioles occurred, and gall-like growths at the nodes often formed. Terminal growth often ceased and the plants appeared to be "frozen," since growth in length ceased. The plants were dead and withered 21 days after treatment. Plants treated with an intermediate amount (0.01 gm. per sq. yd.) manifested intermediate symptoms.

For each treatment four pots of two plants each were used. At time of treatment four pots of plants were harvested and measurements made. Five, 10, 15, 21, and 31 days after treatment, sets of treated and one set of control plants were harvested. The stages of development of control plants at the various times of harvest were as follows:

TIME HARVEST*	STAGE DEVELOPMENT OF CONTROLS
0†	First trifoliate leaf expanding
5	Second trifoliate leaf expanding
10	Third trifoliate leaf expanding
15	Flowers opening; four trifoliate leaves
21	Five trifoliate leaves
31	Pods formed

* Days after treatment.
† Time of treatment.

[1] Work conducted at Camp Detrick, Frederick, Md., from October, 1944, to December, 1944, under the direction of Dr. A. G. NORMAN.

At all harvests several linear measurements were made, the plant was sectioned, and the various parts weighed. The fresh tissues were then dried in an oven for 3 days at about 70° C. to obtain dry weights. Length measurements were the average of the two plants in each pot, and the weights were the sum of the two plants. With few exceptions, length measurements were made of the following parts: total height of plant (to growing point), length of petiole of second trifoliate leaf, and length and width of middle leaflet of second trifoliate leaf. Weights were taken of hypocotyl, first internode, primary leaf blades, petioles of primary leaves, stem above second node (without leaves), trifoliate leaf blades, petioles of trifoliate leaves, roots (plus hypocotyl below soil), and pods. By adding the weights of the appropriate parts, the total weights of various plant portions were obtained.

1. HYPOCOTYL AND FIRST INTERNODE.—Treatment with the growth regulator caused these plant parts to increase greatly in diameter. Their increase in fresh weights was due largely to increase of water content of the tissue (figs. 1, 2). Figures 1 and 2 indicate that the first internodes of treated plants did increase in dry weight but that hypocotyls showed no such increase. It has been shown that stem proliferation is largely due to formation of meristematic tissues, which have little dry weight (2). Decrease in dry weight of hypocotyls of treated plants may be due to cell degeneration.

2. PRIMARY LEAF BLADES.—Primary leaves of treated plants remained green and were still attached to the plant long after primary leaves of control plants had withered and fallen (fig. 3). Exceptions were leaves of plants receiving the highest level (0.1 gm. per sq. yd.) of the growth substance, as these plants were dead and withered 21 days after treatment. Figure 3 indicates that primary leaves of control plants have mostly withered and defoliated 21 days after treatment. The dry weights of primary leaves when plotted showed the same

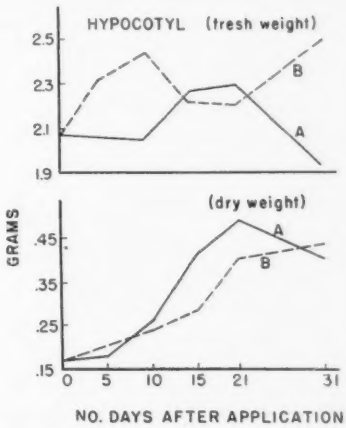


FIG. 1.—Average fresh and dry weights on a two-plant basis of hypocotyls of control plants (*A*) and of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rate of 0.01 gm. per square yard (*B*).

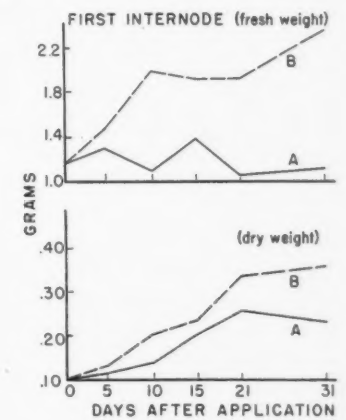
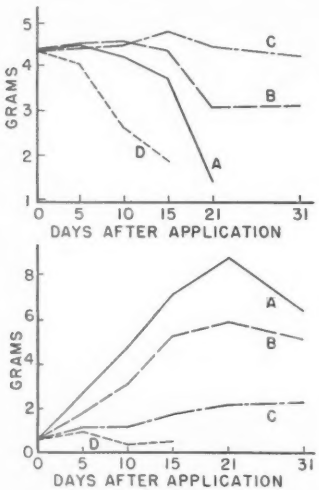


FIG. 2.—Average fresh and dry weights on a two-plant basis of first internodes of control plants (*A*) and of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rate of 0.01 gm. per square yard (*B*).

trend as fresh weights, and hence are not presented.

3. TRIFOLIATE LEAF BLADES, TRIFOLIATE LEAVES, AND WEIGHT OF PLANT ABOVE SECOND NODE.—Treatment was followed by a lowered fresh weight of trifoliate leaf blades, and there was a close correlation between level of growth



FIGS. 3, 4.—Fig. 3 (above), average fresh weight on a two-plant basis of primary leaf blades of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control. Fig. 4 (below), average fresh weight on a two-plant basis of trifoliate leaf blades of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control.

regulator applied and decrease in weight of trifoliate leaf blades produced (fig. 4). A final decrease in weight of trifoliate leaf blades of controls and plants treated with the lowest level of the compound was due to loss of lower leaves by withering and defoliation. Statistical analysis showed that differences in fresh weight of trifoliate leaf blades in controls and treated plants, and of plants treated by different levels of the compound, were highly significant 10 days after treatment. Fresh-weight determinations on trifoliate leaves gave results very similar to those on trifoliate leaf blades. Graphs of weights of trifoliate leaf blades or leaves on a dry-weight basis showed trends very similar to those plotted on a fresh-weight basis.

In a study of the relative inhibitory effects of growth regulators on young red kidney beans, the fresh weight of trifoliate leaf blades or leaves which are produced would appear to be a sensitive criterion. Many growth regulators cause proliferations and swellings on stems which may greatly increase the fresh-plant weight. However, such weight increases may not be indicative of plant recovery. The fresh weight of that part of the plant produced above the second node has been used as a measure of growth inhibition or stimulation in red kidney bean (1), and the results of the present experiment confirm its value for this purpose. Ten days after treatment there were significant differences between controls and treated plants, and between treated plants, when the fresh weight of plants above the second node was used as a criterion. The plotted data have the same general trend as those of figure 4.

Measurements of the length and width of the middle leaflet of the second trifoliate leaf, which was the first leaf produced after treatment, indicate that the growth regulator inhibited leaf expansion in proportion to the amount used. Figure 5 shows the progressive growth in length of the leaflet. Growth in width closely paralleled length.

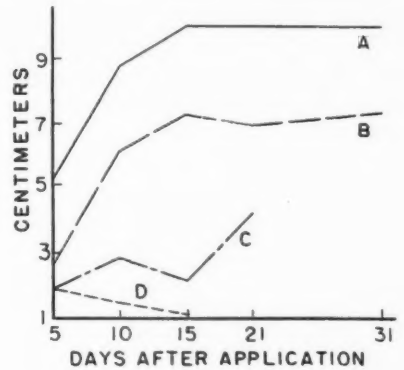
Lengths of petioles of the second trifoliate leaves were also measured. The lowest rate of treatment had little effect on growth in length of the petioles, but the two higher levels greatly decreased it (fig. 6).

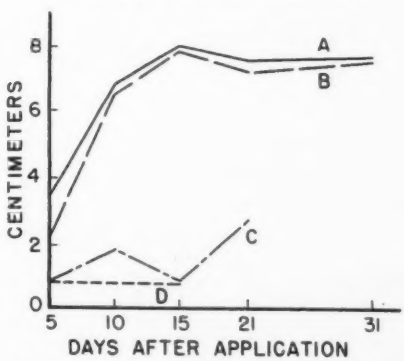
4. TOPS.—The fresh weights of tops were decreased by treatment with the growth regulator. Figure 7 indicates that the differences between controls and treatments and between different treatments were often small. This may be because the greatly increased weight of treated plants was due to proliferated hypocotyls, first internodes, and stems.

Heights of plants were measured from the soil to the growing point. In general, 2,4-dichlorophenoxyacetic acid decreased plant heights, but at 10 days after treatment there was little difference between plants treated with 0.001 and with 0.01 gm. per sq. yd. of agent (fig. 8).

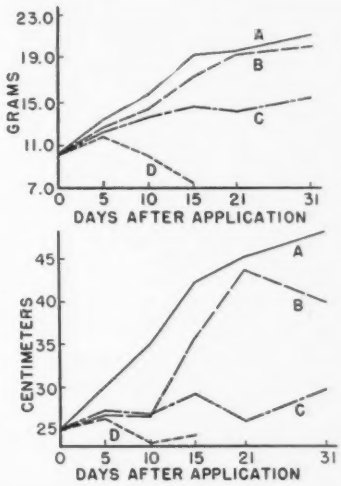
5. ROOTS (INCLUDING HYPOCOTYL BELOW SOIL SURFACE).—Figure 9 indicates that the compound, at rates of 0.1 and 0.01 gm. per sq. yd., decreased the amount of roots on a dry-weight basis, but that the lowest level (0.001 gm. per sq. yd.) had little effect.

6. SHOOT-TO-ROOT RATIO.—There was little effect on shoot-to-root ratio when sublethal amounts of the compound were used (0.001 or 0.01 gm. per sq. yd.). The ratio gradually increased from about 2.7 at time of treatment until a maximum of about 3.7 was reached 21 days after treatment.





FIGS. 5, 6.—Fig. 5 (above), average length of the middle leaflet of the second trifoliate leaf of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rate of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control. Fig. 6 (below), average length of the petiole of the second trifoliate leaf of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control.



FIGS. 7, 8.—Fig. 7 (above), average fresh weight on a two-plant basis of tops of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control. Fig. 8 (below), average height of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control.

7. PODS.—The growth regulator decreased and delayed pod production (table 1). After 31 days, controls produced about 2.8 times more pods on a fresh-weight basis than did plants treated with 0.001 gm. per sq. yd. of the compound. However, when pod development

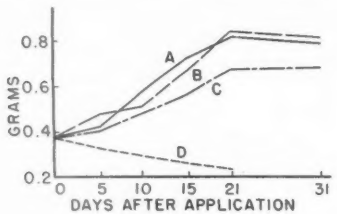


FIG. 9.—Average dry weight on a two-plant basis of roots (plus hypocotyl below soil surface) of red kidney bean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control.

TABLE 1

FRESH WEIGHT (GM.) OF PODS OF RED KIDNEY BEANS AT THREE STAGES OF DEVELOPMENT

TREATMENT (GM./SQ. YD.)	DATE OF HARVEST (DAYS AFTER TREATMENT)		
	21	31	40
Control	0.24	7.29	8.54
0.001	0.02	2.56	6.30
0.01	0	0.2	2.40
0.1	0	0	0

was allowed to continue 9 more days, controls produced only about 1.4 times the weight of pods as treated plants.

EXPERIMENT II: SOYBEAN

Illini soybeans were planted in 4- and 6-inch pots and before treatment were thinned to three plants per pot. Plants were treated on October 17, 1944 (20 days after planting), when the second trifoliate leaves were expanding.

The method of treatment was similar to that of experiment I, except that no Carbowax was used in the solutions. The growth of plants treated with 0.1 gm. per sq. yd. was severely inhibited, while plants treated with 0.001 gm. seemed only slightly affected. Plants treated with 0.01 gm. per sq. yd. were moderately inhibited. Treated soybeans manifested symptoms similar to those of treated kidney beans.

At the time of treatment, and at 7, 11, 16, and 21 days after treatment, plants in 4-inch pots were harvested. Thirty-four and 41 days after treatment, plants in 6-inch pots were harvested. The stage of growth of control plants at the various harvests were:

TIME OF HARVEST*	STAGE OF DEVELOPMENT OF CONTROLS
0†	Second trifoliate leaf expanding
7	Third trifoliate leaf expanding
11	Fourth trifoliate leaf expanding
16	Flowers forming
21	Flowering; five trifoliate leaves
34	Fruiting initiating
41	Many pods formed

* Days after treatment.
† Time of treatment.

At all harvests the same length and weight measurements were taken as in experiment I. All weight figures are means of four replicate pots on a three-plant basis, and length measurements are the means of twelve plants.

1. HYPOCOTYL AND FIRST INTERNODE.—The 2,4-dichlorophenoxyacetic acid caused these parts to increase greatly in diameter. The resultant fresh-weight increase was due, as in case of kidney beans, largely to increased water content of the tissues (fig. 10). For example, 11 days after treatment hypocotyls of control plants and plants sprayed by the compound at 0.01 gm. per sq. yd. each gave dry weights of 0.17 gm., but their fresh weights were 0.84 and 1.21 gm., respectively.

2. PRIMARY LEAVES.—Primary leaves of treated plants became heavier, but those of controls were little changed (fig. 11). The results are similar to those obtained with kidney bean. Graphs based on dry weights were similar to figure 11.

3. TRIFOLIATE LEAF BLADES, TRIFOLIATE LEAVES, AND WEIGHT OF PLANT ABOVE SECOND NODE.—The total fresh weight of leaf blades of treated plants was less than that of control leaf blades, and there was close correlation between decrease in weight and increase in amount of compound used (fig. 12). When fresh weights of trifoliate leaves were plotted, the same general relation existed between fresh weights and amount of compound used; but at certain harvests there were no differences in controls and plants treated with 0.001 gm. per sq. yd., probably because the petioles of treated plants swelled and increased the total leaf weights. Fresh weights of plants above the second node also gave good correlation between decreased weights and increasing amounts of compound. However, differences in weights were often small. In general, data based on a dry-weight basis showed trends similar to those shown in figure 12.

This experiment indicates that fresh weight of trifoliate leaf blades would be the best criterion in a study of inhibitory effects of a growth regulator on soybeans. Twenty-one days after treatment there were significant differences between controls and treated plants and between plants that received various amounts of compound, when fresh weight of leaf blades was used as a criterion. This was

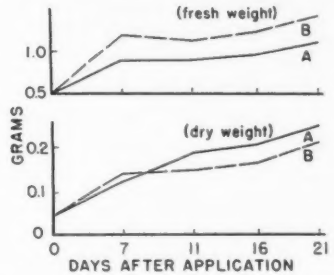


FIG. 10.—Average fresh and dry weight of hypocotyls of controls and soybean plants sprayed with 2,4-dichlorophenoxyacetic acid at the rate of 0.1 gm. per square yard, *B*. Control, *A*. Figures are on a three-plant basis.

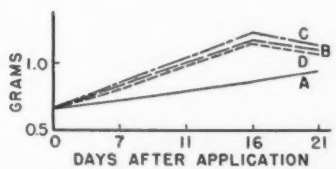
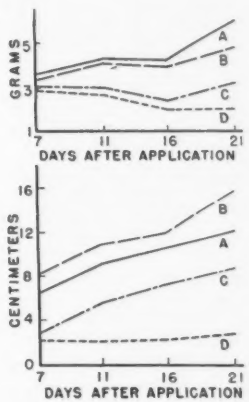


FIG. 11.—Average fresh weight on a three-plant basis of primary leaves of soybean after application of 2,4-dichlorophenoxyacetic acid, at rates of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control.



FIGS. 12, 13.—Fig. 12 (above), average fresh weight on a three-plant basis of trifoliate leaf blades of soybean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control. Fig. 13 (below), average length of the petiole of the second trifoliate leaf of soybean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control.

not true for any other of the criteria studied.

The lengths of the middle leaflets of the second and third trifoliate leaves were less in treated plants, and the higher the treatment rate used, the shorter were the leaflets. However, the length of the petiole of the second trifoliate leaf was increased by the lowest level of the compound, although the two higher levels inhibited growth in length (fig. 13).

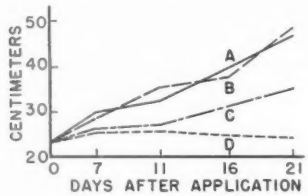
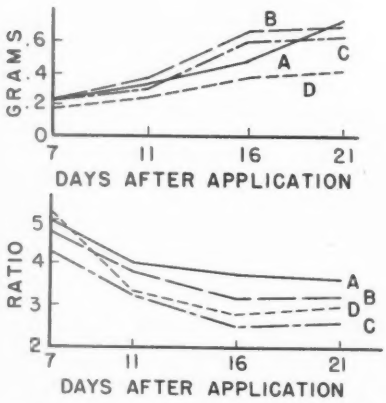


FIG. 14.—Average height of soybean, as measured from soil to the growing point, after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control.



FIGS. 15, 16.—Fig. 15 (above), average dry weight on a three-plant basis of roots (plus hypocotyl below soil surface) of soybean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control. Fig. 16 (below), average ratio (dry-weight basis) of shoot to root of soybean after application of 2,4-dichlorophenoxyacetic acid at rates of 0.001 gm. (*B*), 0.01 gm. (*C*), and 0.1 gm. (*D*) per square yard. *A*, control.

4. TOPS.—The total weight of tops was decreased by application of the growth regulator. However, for 16 days after treatment there was little difference in weight of controls and of plants treated at 0.001 gm. per sq. yd. Plant height was little affected by the smallest amounts of the compound, but length was inhibited by higher rates of application (fig. 14).

5. ROOTS (INCLUDING HYPOCOTYL BELOW SOIL SURFACE).—Dry weights of roots of plants treated with the two smaller amounts of the compound significantly increased when measured 16 days after treatment (fig. 15). After 21 days dry weights of roots of control plants had increased and were about the same as those of plants that received the two lowest treatment levels.

TABLE 2

FRESH WEIGHT (GM.) OF PODS OF SOYBEANS AT TWO STAGES OF DEVELOPMENT

TREATMENT (GM./SQ. YD.)	DATE OF HARVEST (DAYS AFTER TREATMENT)	
	34	41
Control	3.70	7.70
0.001	1.76	6.41
0.01	0.56	3.26
0.1	0.03	0.52

6. SHOOT-TO-ROOT RATIO.—On a dry-weight basis this was decreased by all treatments (fig. 16). Plants treated with the largest amount had a greater such ratio initially than had the control plants, but this soon fell below the control.

7. PODS.—Production was decreased and delayed by the growth regulator (table 2). The delay in pod formation is illustrated by the fact that 34 days after treatment the controls produced 2.1 times the weight of pods of plants treated

with 0.001 gm. of compound per sq. yd. but that this ratio was only 1.2 at the harvest 41 days after treatment.

Summary

1. Plots containing young red kidney bean and soybean plants were sprayed with 2,4-dichlorophenoxyacetic acid at rates of 0.1, 0.01, and 0.001 gm. per sq. yd. The effects of the agent on the growth of various plant parts were studied. Linear, and fresh and dry weight measurements were obtained at several intervals following treatment.

2. Hypocotyls and first internodes increased greatly in diameter. The increase in fresh weight was due mostly to increased water content.

3. Primary leaves of treated plants were much heavier than those of controls. Those of red kidney beans remained green and attached to the plants long after those of controls had withered and fallen.

4. Total weights of trifoliate leaf blades, trifoliate leaves, and weight of plants above second node were decreased by the growth regulator. There was good correlation between decrease in weight and increase in amount of compound applied. These criteria would be excellent in studying the relative inhibitory or stimulating actions of growth regulators on red kidney beans. Ten days after treatment there were highly significant differences between controls and treated plants, and between treated plants, when fresh weights of trifoliate leaves or leaf blades were used as criteria. The differences were significant when the fresh weight of kidney bean plants above the second node was used as a criterion. With soybeans, only fresh weight of trifoliate leaf blades gave significant differences between controls and treated plants and between plants that received various rates of compound when plants were harvested 21 days after treatment.

5. The growth in length and width of certain leaflets which developed after treatment was inhibited by the growth regulator. The two higher levels of compound (0.1 or 0.01 gm. per sq. yd.) inhibited the linear growth of certain petioles. The lowest level (0.001 gm. per sq. yd.) stimulated growth in length of a soybean petiole but had little effect on a bean petiole.

6 The fresh weight of tops was decreased by the growth regulator. Heights were usually decreased but differences caused by various treatments were sometimes small.

7. The compound at rates of 0.01 and 0.1 gm. per sq. yd. decreased dry weight of roots of kidney bean, but 0.001 gm. had little effect. With soybeans the two lower rates of application significantly increased weight of roots at 16 days after treatment. However, by 21 days the weight of control roots was about the same as that of the treated plants.

8. With kidney beans there was little effect on the shoot-to-root ratio when two lower amounts of the compound were used. With soybeans, the ratio was reduced by all treatments.

9. The growth regulator delayed the onset and decreased the amount of pod formation.

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INFLUENCE OF RAINFALL ON THE EFFECTIVENESS OF 2,4-DICHLOROPHENOXYACETIC ACID SPRAYED FOR HERBICIDAL PURPOSES[1]

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Introduction

Since it has been shown that several hours are required for plants to absorb maximum amounts of 2,4-dichlorophenoxyacetic acid sprayed on their leaves (3), an investigation was undertaken to determine to what extent heavy rainfall would remove this compound and reduce its effectiveness as a herbicide. These studies were conducted by means of greenhouse and field experiments in which either aqueous or oil solutions of the compound were sprayed on the plants, which were thereafter exposed at various intervals to heavy artificial rainfall.

Experimentation

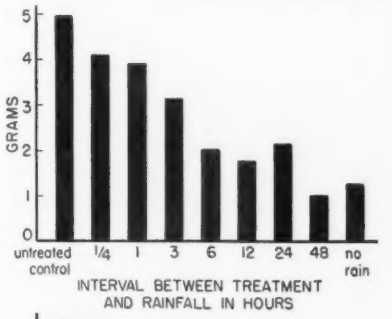
Greenhouse trials

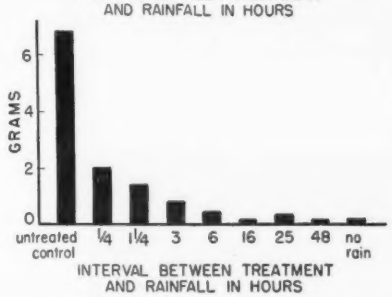
Three experiments were conducted in the greenhouse, one with soybean and two with red kidney bean. All plants were grown in 4-inch pots. Before treatment with 2,4-dichlorophenoxyacetic acid or its ammonium salt, the soybeans were thinned to three and red kidney beans to two per pot; at time of treatment the soybeans had expanded two trifoliate leaves and the red kidney beans, one. Three replicate pots were used for each test. The herbicide was applied as a spray, using 200 ml. air-pressure pumps or a De Vilbiss atomizer. Artificial rainfall of 1 inch was applied in about 5 minutes with a garden-hose nozzle. The water spray was directed upward so that drops of medium size fell almost vertically.

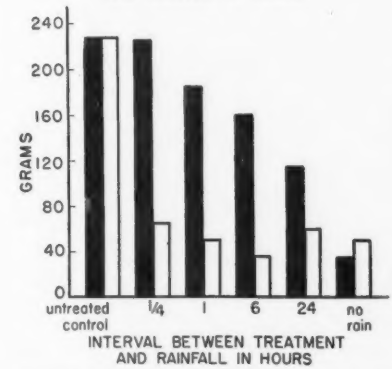
Experiment I.—Young soybean plants were sprayed with an aqueous solution of the ammonium salt of 2,4-dichlorophenoxyacetic acid, or with a solution of the acid in oil, in the amount of 0.1 gm. per square yard of plot surface, in 50 ml. of solution. Spraying was done with 200-ml. pressure pumps. The oil emulsion was prepared by dissolving 1 gm. of the herbicide in 50 ml. of diethylene oxide (Dioxan) and adding 450 ml. of no. 2 diesel fuel oil. Plants were subjected to artificial rainfall at intervals of $\frac{1}{4}$, 1, 3, 6, 12, 24, and 48 hours after treatment. Some treated plants received no artificial rainfall.

All plants sprayed by the herbicide in oil were dead and withered several days after treatment, whether subjected to artificial rainfall or not. Plants sprayed with the aqueous solution were harvested 3 weeks after treatment, when the controls had four or five trifoliate leaves and were fruiting. The fresh weight of trifoliate leaf blades was obtained, as it has been shown that this is an excellent criterion to measure differences in growth inhibition caused by growth regulators of this type (2). The average fresh weights of trifoliate leaf blades on a three-plant basis for control plants and for plants subjected to artificial rainfall $\frac{1}{4}$, 1, 3, 6, 12, 24, and 48 hours after treatment and for treated plants not subjected to rainfall were 4.93, 4.14, 3.84, 3.14, 2.01, 1.66,

[1] Work conducted at Camp Detrick, Frederick, Md., from March, 1945, to September, 1945, under the direction of Dr. A. G. Norman.







FIGS. 1, 2, 3.—Fig. 1 (upper), average fresh weight on a 3-plant basis of trifoliate leaf blades of soybean sprayed by the ammonium salt of 2,4-dichlorophenoxyacetic acid in water at rate of 0.1 gm. per square yard and subjected to rainfall at various intervals after treatment. Fig. 2 (middle), average fresh weights on a 2-plant basis of trifoliate leaf blades of red kidney bean sprayed with the ammonium salt of 2,4-dichlorophenoxyacetic acid in water at rate of 0.1 gm. per square yard and subjected to rainfall at various intervals after treatment. Each treatment is the average of three replicate pots. Fig. 3 (lower), influence of rainfall on effectiveness of ammonium 2,4-dichlorophenoxyacetate in aqueous (black) and of the acid in oil (white) solution upon yield of soybeans. Figures are mean yields per plot (28 row feet) on air-dry basis.

2.17, 0.97, and 1.24 gm., respectively (fig. 1). Only control plants were fruiting, and, in general, the longer the time interval between treatment and rainfall, the less was the recovery made. Figure 1 indicates that plants subjected to rain ¼ hour after treatment were markedly stunted, and that recovery was slight when 6 hours or more had elapsed before treated plants were subjected to rain.

EXPERIMENT II.—Young red kidney bean plants were sprayed with the ammonium salt of 2,4-dichlorophenoxyacetic acid in aqueous solution or with the acid form of this compound dissolved in oil at a rate of 0.1 gm. per square yard, in 10 ml. of liquid. The oil solution was prepared by dissolving 1 gm. of the herbicide in 2 ml. of tributylphosphate and then bringing the solution to 100 ml. volume with no. 2 diesel fuel oil (1). One-fourth, 1¼, 3, 6, 16, 25, and 48 hours after treatment, plants were subjected to artificial rainfall. The plants were harvested 12 days after treatment, when the controls had four trifoliate leaves. The results were similar to those of experiment I. All plants sprayed with the oil solution died several days after treatment, irrespective of the interval elapsing before being subjected to artificial rain. The average fresh weights of trifoliate leaf blades on a two-plant basis for controls and for plants receiving rain ¼, 1¼, 3, 6, 16, 25, and 48 hours after being sprayed by the aqueous herbicidal solution, and for treated plants receiving no rain, were 7.06, 1.86, 1.40, 0.96, 0.44, 0.11, 0.25, 0.10, and 0.14 gm., respectively (fig. 2).

EXPERIMENT III.—This experiment with red kidney beans was similar to experiment II, except that a lower rate of application of herbicide (0.01 gm. per square yard) was used. In general, results were in agreement with those obtained in previous experiments. Unless

rainfall followed almost immediately after application of the aqueous spray, little recovery was made (fig. 4). Oil-treated plants made no recovery.

FIELD TRIAL

On May 1, 1945, Chief soybeans were drilled in rows 18 inches apart. At time of treatment (July 13), plants were in the early flowering stage and 24–30 inches in height. They were sprayed with an aqueous solution of the ammonium salt of 2,4-dichlorophenoxyacetic acid or an oil solution of the acid form at the rate of 0.1 gm. in 10 ml. of solution per square yard of plot. Spray was applied with a De Vilbiss spray gun. The no. 2 diesel-oil solution contained 2% tributylphosphate. The sprays were applied at about 9:30 A.M. and the weather was clear and sunny.

At intervals of $\frac{1}{4}$, 1, 6, and 24 hours after treatment, plants were subjected to a $\frac{1}{2}$-inch artificial rainfall delivered in a

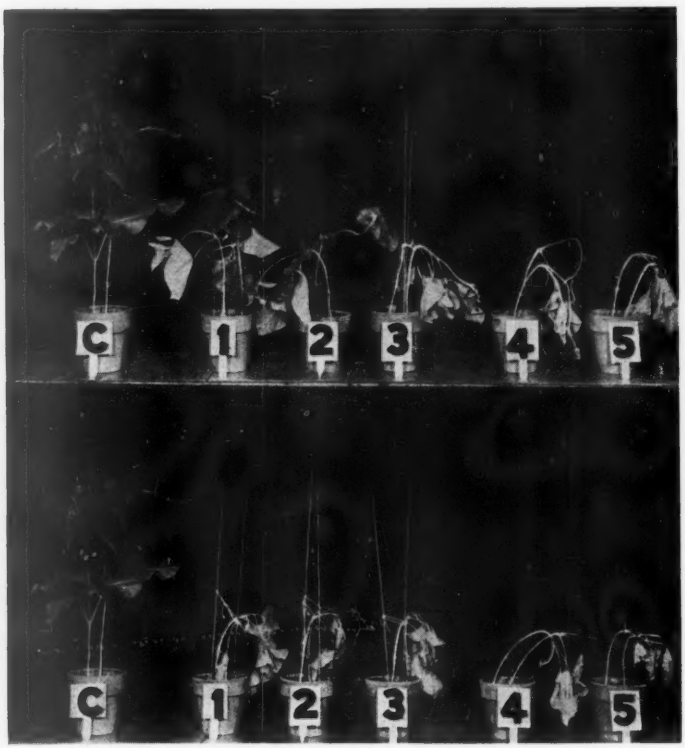


FIG. 4.—Red kidney beans 10 days after treatment with an aqueous solution of ammonium salt of 2,4-dichlorophenoxyacetic acid (*upper*) and with the acid form of this compound in oil (*lower*). *C*, control. Plants in pots 1, 2, 3, and 4 received artificial rain $\frac{1}{4}$, 1, 3, or 12 hours after treatment. Plants in pot 5 received no rain. Some plants treated with aqueous solution made partial recovery, but all plants treated with oil solution were almost dead.

period of about 13 minutes. Additional plants treated with herbicide received no such rainfall. Four-gallon pump-type fire extinguishers were used to produce the rain. A driving spray was secured when the jet of water struck the baffle attached near the nozzle. A heavy rain was thus produced which was probably more severe than most that occur naturally. Eight hours after the 24-hour period had elapsed a natural rain began, and in a period of about 7 hours 3.3 inches fell.

Plots consisted of four rows 7 feet long and 18 inches apart. All treatments were in quadruplicate, and plots were randomized so that the results could be treated statistically.

Soybeans were harvested September 27, 1945, 76 days after treatment. The pods were threshed and air-dry weights of seeds obtained. The data in table 1 indicate that when this herbicide is applied in oil, an immediate violent rainstorm would cause little if any diminution in its effectiveness (fig. 3). However, when aqueous sprays are applied, early rainfall decreases the effectiveness of the herbicide, and the longer the interval between treatment and rain, the more effective is the herbicide. This experiment indicates that any rain falling within 24 hours of treatment is likely to reduce to some extent the effectiveness of aqueous sprays.

TABLE 1

EFFECT OF RAINFALL ON SOYBEANS SPRAYED WITH AQUEOUS SOLUTION OF AMMONIUM SALT OF 2,4-DICHLOROPHENOXYACETIC ACID OR THE ACID IN OIL SOLUTIONS (FIGURES ARE MEAN YIELDS OF BEANS IN GRAMS PER PLOT OF 28-ROW FEET)*

Carrier	Untreated control	Treated plants				
		Interval between treatment and rainfall (hours)				
		½	1	6	24	No rain
Aqueous	227.8	226.8	191.8	160.3	116.5	35.0
Oil	.....	63.3	49.8	35.5	61.8	49.5

* Significant difference between means at 5% is 54.33 gm. Highly significant difference between means at 1% is 73.41 gm.

Discussion and Summary

1. The influence of artificial rainfall on the effectiveness of 2,4-dichlorophenoxyacetic acid in oil or an aqueous solution of its ammonium salt sprayed for herbicidal purposes was studied. In greenhouse experiments, young soybean and red kidney bean plants were used, and in the field soybean plants at early flowering stage.

2. When the growth regulator was applied in oil solution, an immediate heavy rain caused no diminution in the response to the herbicide, as drops of rain were apparently shed from the oil-covered leaves without removal of the oil solution. When aqueous solutions of the compound were used, rainfall often decreased plant response (inhibition). In greenhouse experiments, the plant responses were not decreased by an artificial rainfall if 6 hours or more elapsed between application of the compound and artificial rain, but in a field trial rainfall occurring within 24 hours of application reduced effectiveness of the herbicide. This difference in effect of rain is attributed to the varying developmental stages of test plants (4) or to dissimilar environmental conditions of greenhouse and field.

3. The use of an oil carrier for a herbicide may be advantageous in regions of frequent heavy rainfall. Since all artificial rains in these experiments were much heavier than natural rainfall, trials using lower intensities of simulated rain would be of value when aqueous sprays of growth regulators are applied.

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QUANTITATIVE ASPECTS OF AQUEOUS-SPRAY APPLICATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID FOR HERBICIDAL PURPOSES[1]

HAROLD H. SMITH, LT., U.S.N.R.

Introduction

The use of sprays containing growth-regulating compounds for herbicidal purposes is a field of increasing importance, and it is desirable to obtain information on the relation between some of the characteristics of the spray and the plant response. The purpose of these experiments was to investigate certain features, such as volume, concentration, amount of compound, droplet size, and degree of interception, and to study the extent to which they influence the growth-inhibiting quality of the sprays.

TABLE 1

SETTINGS OF THE DEVILBISS SPRAY GUN AND DROPLET SIZE IN THE AQUEOUS SPRAYS DELIVERED

SETTING NO.	FLUID NEEDLE ADJUSTMENT (TURNS)	PRESSURE (POUNDS PER SQUARE INCH)	DROPLET SIZE	
			Av. diameter (μ)	Coeff. var. (per cent)
1.....	0	10	561 ± 373	66.4
2.....	0	20	250 ± 138	55.2
3.....	6	10	70 ± 38	54.5
4.....	8	40	30 ± 14	49.6

[1] Studies conducted at Camp Detrick, Frederick, Md., from August, 1944, to February, 1945, under the supervision of Dr. A. G. Norman.

Methods

Sprays were applied with a CV-type DeVilbiss paint-spray gun, fitted with a no. 90 air cap and *F* fluid tip and needle. Four combinations of adjustments and pressure were used to produce different types of sprays (table 1).

The apparatus was calibrated, in order that known volumes of spray of definite droplet characteristics could be delivered per unit of time. A chamber with a basal area of 196 square inches and sufficiently tall to permit making applications from a height of 4 feet was used for all spray treatments.

Results

VOLUME-CONCENTRATION STUDIES

Differential responses of plants to treatments with a constant amount of the ammonium salt of 2,4-dichlorophenoxyacetic acid applied in aqueous

sprays of different volume and concentration were studied. The experimental plants used were kidney beans grown in the greenhouse to the stage of development at which the first trifoliate leaf was beginning to unfold. The compound was applied at a rate of 2.5 mg. per square yard, which was sufficient to produce threshold effects. Green weight of leaf blades of new growth, 17 days after treatment, was the criterion used for measuring plant response. Each treatment was replicated four times.

The results, arranged in table 2, showed that all treatments produced significant inhibition of growth compared with the control. The spray was most effective when applied in a volume of 10–20 ml. per square yard and a concentration of 125–250 p.p.m. There was no significant difference in effect between these two volume-concentration rates or between treatments at 40 and 100 ml. per square yard. The latter may be considered a spray of "saturation" volume. However, the 20 ml. per square yard rate was more effective than 40 ml., 10 ml. more effective than 4 ml., and 4 ml. more effective than 2 ml. (fig. 1).

Growth inhibition was progressively greater as concentration was increased, and volume decreased from saturation levels to 10–20 ml. per square yard, but there was a rapid decrease in effectiveness of the spray with further reductions in volume when the amount of compound was kept constant (fig. 2).

TABLE 2

EFFECT OF AQUEOUS SPRAYS OF THE AMMONIUM SALT OF 2,4-DICHLOROPHENOXYACETIC ACID APPLIED TO KIDNEY BEANS AT 2.5 MG. PER SQUARE YARD IN DIFFERENT VOLUME-CONCENTRATION RATES

Spray vol. (ml. per square yard)	Concentration		Green weight of leaves of new growth*	
	(Per cent)	(P.p.m.)	Setting 2 (gm.)	Setting 3 (gm.)
Control	0	0	2.55	2.55
100	0.0025	25	1.35	1.82
40	0.00625	62.5	1.35	1.42
20	0.0125	125	0.59	0.72
10	0.0250	250	0.64	0.40
4	0.0625	625	1.82	0.89
2	0.1250	1250	†	1.74

* Minimum significant difference between means at 5% level of probability is 0.42 gm.

† Volume rate of 2 ml. per square yard could not be delivered at setting 2.

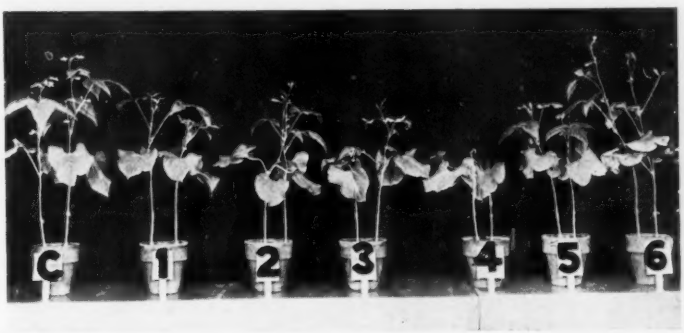


FIG. 1.—Effect of sprays of the ammonium salt of 2,4-dichlorophenoxyacetic acid applied in different volume-concentration rates on kidney beans. Setting 3 of the spray gun. *C*, control; *1*, 100 ml. per square yard; *2*, 40 ml. per square yard; *3*, 20 ml. per square yard; *4*, 10 ml. per square yard; *5*, 4 ml. per square yard; *6*, 2 ml. per square yard.

Further experiments were conducted at low-volume rates in view of the following considerations. Previous work on weed-killing, because of the necessity of getting complete coverage of plants to produce contact injury, has been carried out at high-volume rates, i.e., 100–150 gallons per acre, which is roughly equivalent to 100 ml. per square yard. Compounds of the phenoxyacetic acid type are translocated and systemic in effect and may therefore be effective at far lower-volume rates. Even single droplets will kill young plants. Conceivably, weed-killing could be accomplished by sprays distributed from airplanes if low-volume rates were found to be acceptable. The results reported above indicated that plant responses to low-volume sprays were substantially different in magnitude from those obtained when applications were made in larger volumes.

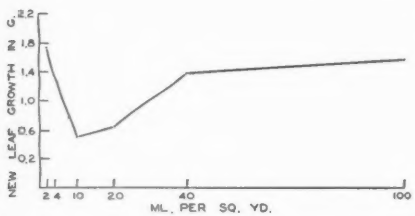


FIG. 2.—Effect of sprays of the ammonium salt of 2,4-dichlorophenoxyacetic acid applied to kidney beans at a constant rate of 2.5 mg. per square yard in different volumes.

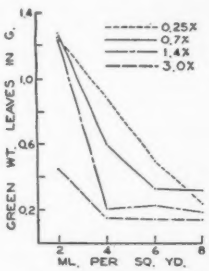


FIG. 3.—Effect of four different concentrations of the ammonium salt of 2,4-dichlorophenoxyacetic acid applied in four low-volume spray rates on soybeans.

TABLE 3

EFFECT OF LOW-VOLUME SPRAYS OF THE AMMONIUM SALT OF 2,4-DICHLOROPHENOXYACETIC ACID ON SOYBEANS

Volume rate (ml. per square yard)	Concentration (per cent)	Weight rate (mg. per square yard)	Leaf weight (gm.)*
2	0.25	5	1.25
	0.70	14	1.28
	1.40	28	1.24
	3.00	60	0.45
4	0.25	10	0.89
	0.70	28	0.60
	1.40	56	0.21
	3.00	120	0.15
6	0.25	15	0.50
	0.70	42	0.33
	1.40	84	0.24
	3.00	180	0.15
8	0.25	20	0.24
	0.70	56	0.32
	1.40	112	0.20
	3.00	249	0.14

* Minimum significant difference between means at 5% level of probability is 0.38 gm.

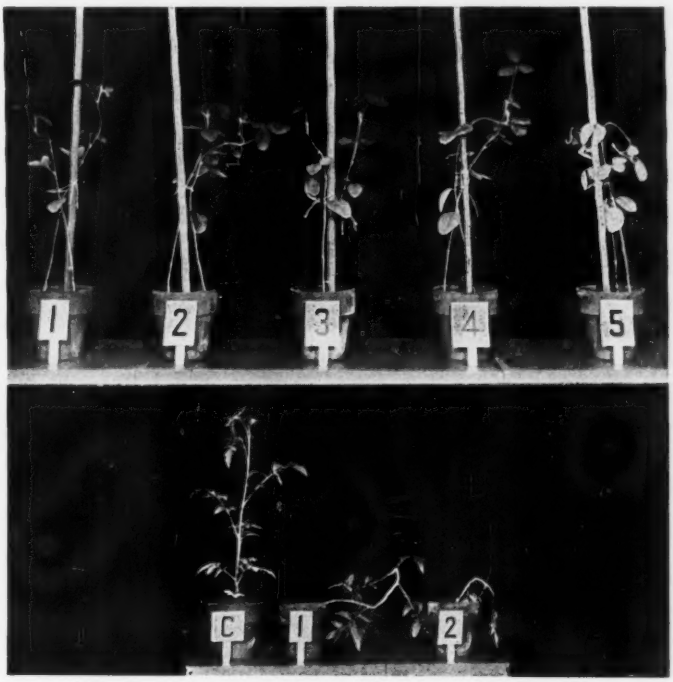
An experiment was set up in which four different concentrations of an aqueous solution of the ammonium salt of 2,4-dichlorophenoxyacetic acid were each sprayed at four different volume rates, namely, 2, 4, 6, and 8 ml. per square yard. Soybean plants with three well-formed trifoliate leaves were used as experimental material, and similar treatments were applied to young tomato plants. Weights of the leaves of the entire plant, 20 days after spraying, were used as the criterion for measuring response.

The results are shown in table 3, arranged graphically in figure 3, and illustrated by figures 4 and 5.

In evaluating the graph (fig. 3) it can

safely be assumed that where the green weight of leaves was 1.2 gm. or more, there was no inhibitory effect, and where the weight was 0.45 gm. or less, complete inhibition or "kill" had been effected.

Similar results were obtained with tomato plants, except that distortion was



FIGS. 4, 5.—Fig. 4, effect of sprays of the ammonium salt of 2,4-dichlorophenoxyacetic acid applied at a volume rate of 2 ml. per square yard on soybeans, 14 days after treatment. *1*, 0.25%; *2*, 0.35%; *3*, 0.70%; *4*, 1.40%; *5*, 3.00%. Only the treatment at 3% was effective in inhibiting growth. Fig. 5, effect of sprays of the ammonium salt of 2,4-dichlorophenoxyacetic acid applied at a rate of 14 mg. per square yard on tomatoes. *C*, control (av. wt., 6.13 gm.); *1*, 2 ml. per square yard, 0.70% (av. wt., 4.26 gm.); *2*, 4 ml. per square yard, 0.35% (av. wt., 1.64 gm.). Note greater effectiveness of the same amount of compound applied in the larger volume.

pound; but a 3 per cent application (60 mg. per square yard) caused complete inhibition. (2) At a 4-ml. per square yard volume rate, a spray of 1.4 per cent concentration (56 mg. per square yard) killed soybeans. A concentration of 0.70 per cent was not sufficiently strong.

accomplished by lighter dosages and the data were less consistent.

The following conclusions were tenable: (1) At a volume rate of 2 ml. per square yard there was no inhibition in growth from spray applications of 0.25–1.40 per cent concentration of com-

(3) In a spray volume of 6 ml. per square yard a concentration of 0.70 per cent (42 mg. per square yard) killed soybeans. At this same volume rate a concentration of 0.25 per cent was not sufficient to cause complete inhibition of soybeans but was highly effective on tomatoes.

(4) Applied in a volume of 8 ml. per square yard at a concentration of 0.25 per cent (20 mg. per square yard), the spray killed both soybeans and tomatoes.

These results showed that in sprays of low-volume rate, as the volume was reduced, progressively more growth-inhibiting substance was required to produce the same response. At least 60 mg. per square yard of the ammonium salt of 2,4-dichlorophenoxyacetic acid was needed to kill young soybean plants with sprays applied at a 2-ml. per square yard rate, and only 20 mg. per square yard at an 8-ml. per square yard volume rate.

Droplet size studies

To study the relative effectiveness of 2,4-dichlorophenoxyacetic acid when applied in aqueous sprays of different droplet size, two settings (no. 1 and no. 4, table 1) of the spray gun were used. When young kidney-bean plants were treated with large- and with small-droplet sprays at three volume rates and three rates of compound, it was found that those composed of the larger droplets were more effective (table 4 and fig. 6). This result was due mostly to a higher percentage of spray interception when the large droplets were used.

The sprays of small-droplet size were found to be more effective when applied in a relatively large volume, such as 60 ml. per square yard (table 4 and fig. 6). The relative ineffectiveness of small-droplet sprays at low volume might be due to the fact that many of the droplets were left "stranded" on islets on the lamina of the leaf without access to conductive tissue. In sprays of large volume there would be a coalescence of droplets into larger ones (as in large-droplet sprays at low volume), which would be more likely to come in contact at some point with conductive tissue, so that all the compound in the drop could be absorbed and transported.

Interception studies

Experiments were carried out to investigate possible relationships between amount of interception of sprays and differences in droplet size and volume rate

TABLE 4

EFFECT OF LARGE AND SMALL-DROPLET AQUEOUS SPRAYS OF 2,4-DICHLOROPHENOXYACETIC ACID ON GROWTH OF KIDNEY BEANS HARVESTED 12 DAYS AFTER APPLICATION

Volume rate (ml. per square yard)	Weight rate (mg. per square yard)	Green weight of new growth*	
		Large drops (gm.)	Small drops (gm.)
60	0.5	5.76	5.36
	1.0	2.88	5.19
	2.0	0.93	4.10
30	0.5	2.89	6.02
	1.0	2.58	5.40
	2.0	0.99	5.28
10	0.5	3.46	6.22
	1.0	2.14	6.79
	2.0	1.16	5.62
Control	0.0	7.76	7.76

* Minimum significant difference between means at 5% level of probability is 1.61 gm.

and further to correlate the findings with plant response.

In order to obtain a measure of the amount of growth-inhibiting substance intercepted by plants exposed to different types of sprays, a method for determining the percentage of recovery of dye solution, applied under the same conditions, was employed. Two stable water-soluble Du Pont dyes were selected, namely, anthraquinone blue and crocein scarlet. A 1 per cent stock solution and nine dilutions, ranging from 1 to 50 mg. per cent, were made up with each dye,

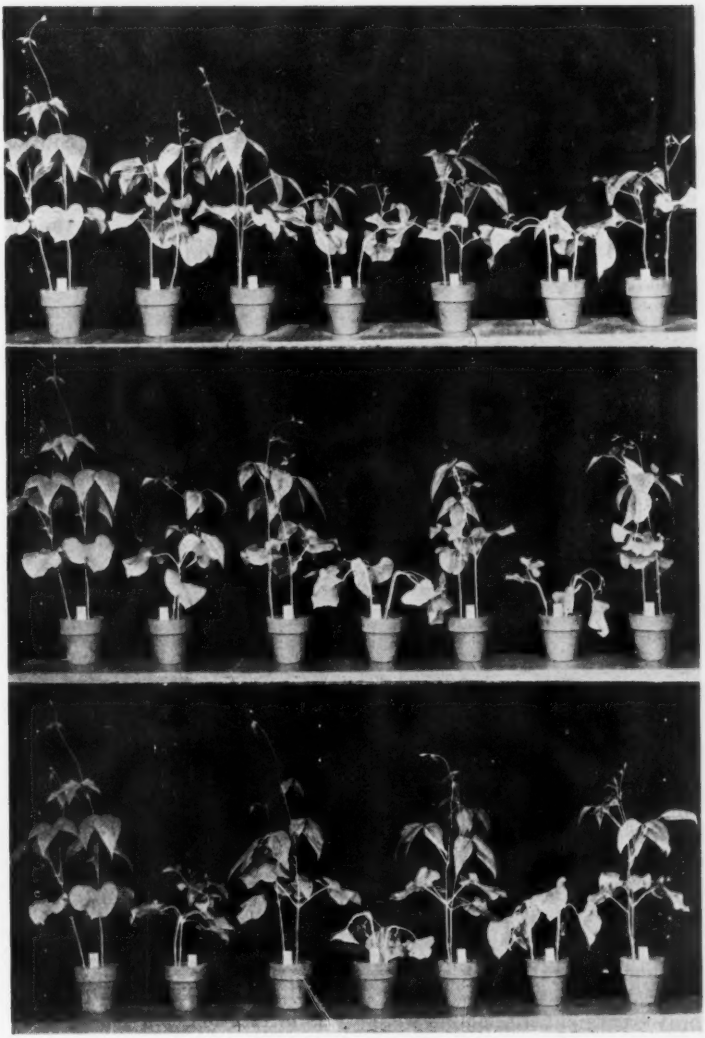


FIG. 6.—Kidney-bean plants treated with 2,4-dichlorophenoxyacetic acid applied in sprays of two different droplet sizes in a volume of 60 ml. per square yard (above), 30 ml. per square yard (center), and 10 ml. per square yard (below). Left to right: Control, 0.5 mg. per square yard (large drops); 0.5 mg. per square yard (small drops); 1.0 mg. per square yard (large drops); 1.0 mg. per square yard (small drops); 2.0 mg. per square yard (large drops); and 2.0 mg. per square yard (small drops).

and standard calibration curves on the Evelyn photoelectric photometer were constructed.

Using three different settings of the gun, the dye solutions were sprayed on soybean plants, the second trifoliate leaves of which were beginning to unfold. The solutions were applied in the same manner as those sprays containing 2,4-dichlorophenoxyacetic acid. Following the spray treatment, the plants were washed immediately in 200 ml. of distilled water, and the concentrations of dye in the different washes were determined. From these figures the per cent interception per plant was calculated. The approximate surface area of each plant was determined by obtaining the leaf area with a planimeter and the stem area by applying the formula for the lateral area of a cylinder to measurements of height and mean width. The interception per square inch, in terms of total volume sprayed, was calculated. The results are shown in table 5.

TABLE 5

INTERCEPTION BY SOYBEAN PLANTS OF DYE SOLUTIONS APPLIED IN DIFFERENT TYPES OF SPRAYS

Spray-gun setting no.	Vol. solution sprayed (ml.)	Plant area (sq. in.)	Concentration of wash (mg. per cent)	Solution intercepted of total volume sprayed	
				Per plant (per cent)	Per square inch (per cent)
1	5	10.77	1.70	6.8	0.68
		7.22	0.50	2.0	0.28
		8.24	1.20	4.8	0.58
2	5	8.06	1.00	4.0	0.50
		7.80	0.95	3.8	0.49
		8.24	1.25	5.0	0.61
4	5	9.42	0.25	1.0	0.11
		7.65	0.20	0.8	0.10
		9.55	0.40	1.6	0.17
4	10	9.22	0.80	1.6	0.17
		10.02	0.60	1.2	0.12
		9.25	0.60	1.2	0.13

In order to obtain a general figure for the average amount of interception per plant, the average surface area for all plants used in this experiment, calculated to be 8.79 square inches, was multiplied by the average per cent interception per square inch for each spray type. These data are shown in table 6.

TABLE 6

AVERAGE INTERCEPTION AND RETENTION OF DYE SOLUTION BY SOYBEAN PLANTS EXPOSED TO DIFFERENT TYPES OF SPRAY

Spray-gun setting no.	Vol. solution sprayed (ml.)	Average rate of interception per square inch (per cent)	Average rate of interception per plant (per cent)
1	5	0.51	4.48
2	5	0.53	4.66
4	5	0.13	1.14
4	10	0.14	1.23

Only about one-fourth as much spray was intercepted and retained per plant in applications composed of small droplets as in those composed of large droplets. The sprays of large-size (*ca.* 561 μ diam.) and intermediate (*ca.* 250 μ diam.) droplets gave essentially the same result, namely, that about $4\frac{1}{2}$ per cent was intercepted per plant. They had in common the characteristic that the drops were large enough to fall directly, whereas the spray of small droplets (*ca.* 30 μ diam.) remained suspended in the air for a while, and some may have been diverted to additional surfaces of the chamber.

Some preliminary experiments were performed to study the relation of spray

volume to degree of interception, using setting 3 of the spray gun and young kidney beans for plant material. The data were corrected for differences in plant size. It was found that, at a volume rate of 100 ml. per square yard, 2.07 per cent of the spray was intercepted per plant; at 10 ml. per square yard, 3.19 per cent; and at 2 ml. per square yard, 1.94 per cent. This provided evidence that some of the differences in plant response correlated with volume of spray (see table 2) could be attributed to a difference in the amount of spray intercepted and retained.

Summary

1. An apparatus was set up, with which sprays of different droplet size could be applied at desired volume rates on plants for the purpose of determining the effect of growth-regulating compounds applied as herbicides.

2. Aqueous sprays, containing a constant threshold amount of the ammonium salt of 2,4-dichlorophenoxyacetic acid, were found to be most effective at a volume rate of 10–20 ml. per square yard when applied to young kidney-bean plants. Rates of 40–100 ml. per square yard were less effective, and there was a progressive decrease in magnitude of response as the volume was reduced below 10 ml. per square yard.

3. Further studies were made on sprays applied at the low rates of 8, 6, 4, and 2 ml. per square yard. It was shown that, as the volume was reduced, a progressively larger amount of growth-inhibiting substance was required to kill young soybean plants. Twenty mg. of compound per square yard was needed at the 8-ml. per square yard volume rate and at least 60 mg. per square yard (3 per cent concentration) at the 2-ml. per square yard rate.

4. Sprays of relatively large-droplet size (561–250 μ av. diam.) were more effective than those of smaller-droplet size (30 μ av. diam.).

5. The investigations on interception showed that more solution was deposited and retained on a plant when sprays of larger-droplet size were used. There was also an indication that a higher percentage of the spray was intercepted and retained when applied at volume rates of 10–20-ml. per square yard than in smaller or larger volumes.

6. For most efficient use of growth-regulating compounds, they should be applied in sprays devoid of finely atomized particles and in volumes of 10–20 ml. per square yard rather than at saturation levels of about 100 ml. per square yard. Rates below 10 ml. per square yard, while less efficient in terms of amount of compound required, are of interest. The fact that growth inhibitors can be effective when applied in such low volumes opens up new fields for large-scale practical application of herbicides.

THE RESPONSE OF KIDNEY-BEAN AND SOYBEAN PLANTS TO AQUEOUS-SPRAY APPLICATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID WITH AND WITHOUT CARBOWAX[1]

W. B. ENNIS, JR., LT., U.S.N.R., AND F. T. BOYD, CAPT., A.U.S.

Introduction

It has been reported (3) from single-droplet tests on kidney-bean plants that Carbowax not only enhanced the inhibitory action of 2,4-dichlorophenoxyacetic acid but also appeared to possess deleterious properties of its own when applied in aqueous sprays. These investigations were undertaken to determine whether the addition of Carbowax to aqueous solutions of 2,4-dichlorophenoxyacetic acid would increase their herbicidal effectiveness when sprayed in relatively low volumes on soybean and kidney-bean plants.

Materials and methods

For the purpose of this investigation three polyethylene glycols, which are commercially available as Carbowax 1500, 1540, and 4000, were used. MITCHELL and HAMNER have discussed the properties of these compounds (3).

PLANT MATERIAL.—Illini soybean and red kidney-bean plants were grown in 4-inch pots containing a fertile greenhouse-soil mixture and were thinned to two uniform plants per pot. When treated, the soybean plants were 17 days old and the red kidney-bean plants 13 days old. Care was exercised in selecting uniform plants for the experiment. The plants were harvested 11 days following spray application.

SOLUTIONS AND METHODS OF APPLICATION.—Thirty ml. aqueous solutions containing 1, 5, and 10 mg., respectively, of 2,4-dichlorophenoxyacetic acid, and similar solutions, to which 0.5% Carbowax 1500, 1540, and 4000 had been added, were used on the soybean plants; for treatment of kidney-bean plants the aqueous and the 0.5% Carbowax solutions contained 0.5, 2.5, and 5.0 mg. per 30 ml. The solutions containing Carbowax were prepared in a manner similar to that described by MITCHELL and HAMNER (3). These concentrations had previously been found to approach threshold concentrations for the test plant at the stage of development indicated above. Both soybean and kidney-bean plants were treated with 0.5% aqueous solutions of the three Carbowaxes for controls.

All solutions were applied at the rate of 30 ml. per square yard with a DeVilbiss paint-spray-gun apparatus under 20 pounds per square inch constant air pressure, which produces a medium-size droplet spray. A 14×14-inch spray chamber was employed, and each of the four pots of plants used in one test was sprayed singly.

CRITERIA OF GROWTH.—A few hours prior to treatment, individual plant measurements were taken of all plants by measuring the length of stem between unifoliate leaf node and node of the youngest trifoliate leaf. The same measurement was taken just prior to harvest, and the difference in these two figures was taken as one measure of growth. Green and dry weights of the plant portion above the unifoliate leaves were also taken as additional criteria of effect.

[1] Studies conducted at Camp Detrick, Frederick, Md., from June, 1944, to December, 1944, under the supervision of Dr. A. G. Norman.

Observations

In the case of kidney-bean plants, increasing degrees of epinasty resulted when increasing amounts of 2,4-dichlorophenoxyacetic acid were applied (fig. 1). When the acid was applied in aqueous solutions containing either 0.5% Carbowax 1500, 1540, or 4000, a greater degree of stem curvature was produced, indicative of a greater degree of plant inhibition, than from equal rates of acid applied in a wholly aqueous spray (fig. 2). Similar effects were obtained by MITCHELL and HAMNER (3) when the droplet test was employed.

On the other hand, the response of soybean plants to the Carbowax sprays differed considerably from that of kidney beans. In figures 2 and 3 it is to be noted that the wholly aqueous sprays of 2,4-dichlorophenoxyacetic acid produced as much epinasty as the corresponding

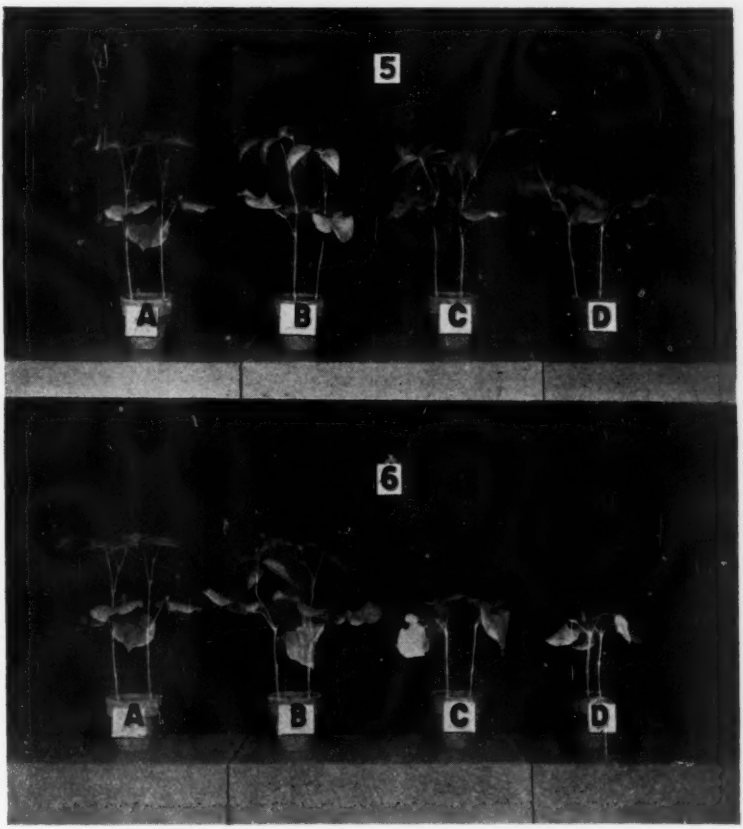


FIG. 1.—Effects of spraying kidney-bean plants with 2,4-dichlorophenoxyacetic acid in wholly aqueous solutions (5, above) and 0.5% Carbowax 1500 solutions (6, below). The volume of spray was 30 ml. per square yard and was applied 13 days after planting. Acid rate per square yard: *A*, carrier control; *B*, 0.5 mg.; *C*, 2.5 mg.; *D*, 5.0 mg. Eleven days after treatment.

FIG. 2.—Effects of spraying kidney-bean (above) and soybean plants (below) with 2,4-dichlorophenoxyacetic acid in wholly aqueous solution and in 0.5% Carbowax solutions. The acid was applied in 30-ml. spray solutions at the rate of 5.0 and 2.5 mg. per square yard, respectively, to the soybeans and kidney beans. *A*, untreated; *B*, water; *C*, Carbowax 1500; *D*, Carbowax 1540; *E*, Carbowax 4000. Eleven days after treatment.

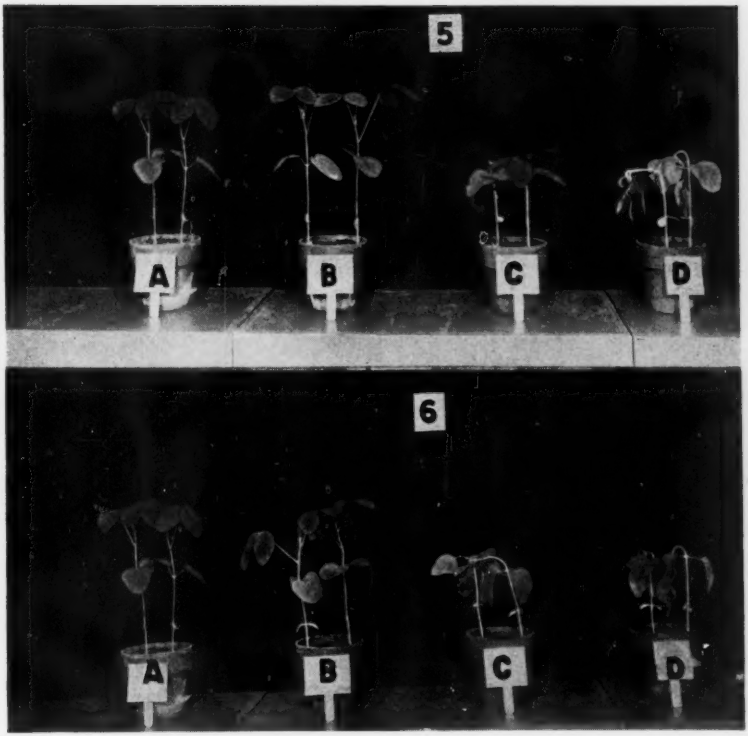


FIG. 3.—Effects of spraying soybean plants with 2,4-dichlorophenoxyacetic acid in wholly aqueous solutions (5, above) and 0.5% Carbowax 1500 solutions (6, below). The volume of spray was 30 ml. per square yard and was applied 17 days after planting. Acid rate per square yard: *A*, control; *B*, 1.0 mg.; *C*, 2.5 mg.; *D*, 10.0 mg. Eleven days after treatment.

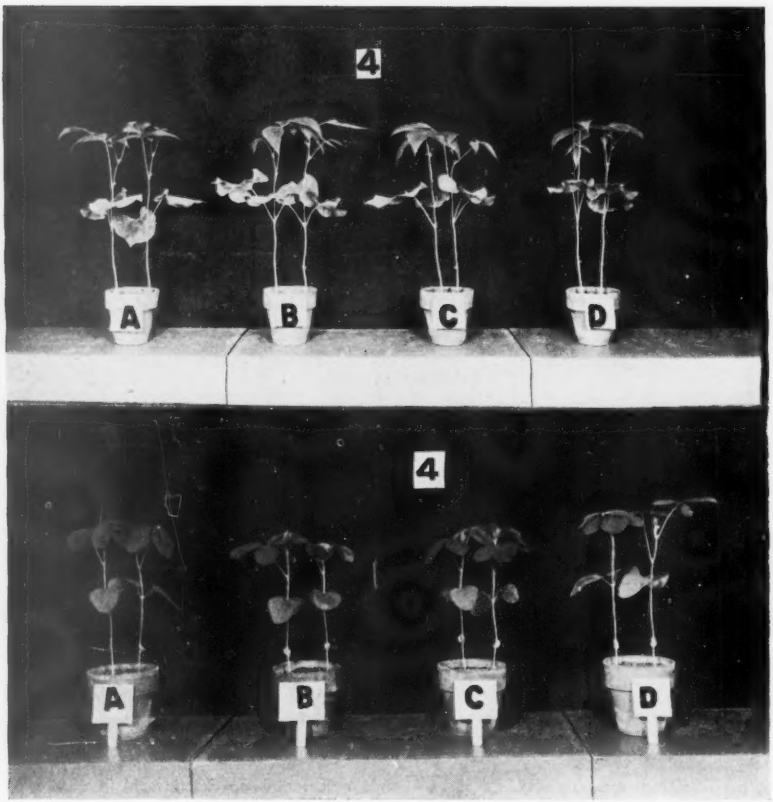


FIG. 4.—Effects of spraying kidney-bean (above) and soybean plants (below) with 0.5% Carbowax solutions at a volume rate of 30 ml. per square yard (each treated at same age as indicated in figs. 1 and 3). *A*, control; *B*, Carbowax 1500; *C*, Carbowax 1540; *D*, Carbowax 4000. Eleven days after treatment.

treatments involving either of the Carbowaxes. Spray applications of 0.5% solutions of the three waxes did not produce any noticeable response in the soybean or kidney-bean plants (fig. 4).

The three criteria of treatment responses were in surprisingly close agreement. It is apparent (tables 1, 2, and 3) that the compound was significantly more effective in producing inhibition of growth in kidney-bean plants when applied at the rate of 2.5 mg. per square yard in Carbowax solutions than when applied in wholly aqueous solutions. When the compound was applied to soybean plants at a rate of 5 and 10 mg. per square yard, no significant difference in the inhibition of growth was produced by the Carbowax sprays, as compared to wholly aqueous sprays (tables 1, 2, and 3).

The general responses of various plant portions of soybean and kidney-bean plants to the acid were essentially the same as have already been described (2, 3).

TABLE 1

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID UPON STEM ELONGATION OF SOYBEAN AND KIDNEY-BEAN PLANTS WHEN APPLIED WITH AND WITHOUT CARBOWAX IN WATER SPRAYS. VALUES ARE MEAN STEM LENGTHS (CM.) BETWEEN UNIFOLIATE LEAF NODE AND YOUNGEST TRIFOLIATE LEAF NODE OF EIGHT PLANTS. FINAL MEASUREMENTS WERE MADE 11 DAYS AFTER TREATMENT*

2,4-DICHLOROPHENOXYACETIC ACID (MG. PER SQUARE YARD)	CARRIER SOLUTIONS			
	Aqueous	0.5% Carbowax 1500	0.5% Carbowax 1540	0.5% Carbowax 4000
	Soybeans			
0.0 (control)	6.1	6.8	7.5	7.3
1.0	6.4	6.5	5.5	3.8
5.0	3.9	3.7	3.3	3.1
10.0	2.8	2.9	3.3	2.4
	Kidney beans			
0.0 (control)	14.7	14.5	12.8	11.3
0.5	14.6	12.8	14.1	11.1
2.5	10.6	5.0	6.1	5.7
5.0	8.2	4.1	5.1	4.2

* Minimum difference between means of soybeans for significance at the 5% level of probability is 0.9 cm., at the 1% level of probability, 1.2 cm. Minimum difference between means of kidney beans for significance at the 5% level of probability is 2.2 cm., at the 1% level of probability, 2.9 cm.

TABLE 2

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID UPON THE GREEN WEIGHT OF SOYBEAN AND KIDNEY-BEAN PLANTS WHEN APPLIED WITH AND WITHOUT CARBOWAX IN WATER SPRAYS. VALUES ARE MEAN GREEN WEIGHTS (GM.) OF VEGETATIVE PORTION ABOVE THE UNIFOLIATE LEAVES OF EIGHT PLANTS*

2,4-DICHLOROPHENOXYACETIC ACID (MG. PER SQUARE YARD)	CARRIER SOLUTIONS			
	Aqueous	0.5% Carbowax 1500	0.5% Carbowax 1540	0.5% Carbowax 4000
	Soybeans			
0.0 (control)	3.58	3.74	3.90	3.78
1.0	3.51	3.38	2.99	2.25
5.0	2.34	2.30	2.49	2.11
10.0	2.19	2.28	2.18	1.80
	Kidney beans			
0.0 (control)	7.08	7.52	7.09	7.09
0.5	7.40	6.85	6.88	5.80
2.5	5.55	2.45	2.04	2.25
5.0	3.71	1.49	1.39	1.45

* Minimum difference between means of soybeans for significance at the 5% level of probability is 0.61 gm., at the 1% level of probability, 0.82 gm. Minimum difference between means of kidney beans for significance at the 5% level of probability is 1.25 gm., at the 1% level of probability, 1.67 gm.

Discussion

It has previously been shown (3) that solutions containing above 9% Carbowax 1500 were toxic to greenhouse-grown tomato plants and that soybean and kidney-bean leaves became yellowish when sprayed with solutions containing 10 and 15% by weight of Carbowax 1500, while lower concentrations of Carbowax did not appear to produce any deleterious effects. Extensive exploratory studies with soybeans have largely corroborated these results. While investigating Carbowaxes in this study it was found that the 1-mg. applications of 2,4-dichlorophenoxyacetic acid appeared consistently to produce greater inhibition of growth of soybeans when applied in 0.5% Carbowax 4000 than in a wholly aqueous spray or in either 0.5% Carbowax 1500 or 1540 (tables 1, 2, and 3). This would suggest that Carbowax 4000 was more effective in some way in accentuating the action of the growth-regulating substance than were Carbowax 1500 or 1540.

TABLE 3

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID UPON THE OVEN-DRY WEIGHT OF SOYBEAN AND KIDNEY-BEAN PLANTS WHEN APPLIED WITH AND WITHOUT CARBOWAX IN WATER SPRAYS. VALUES ARE MEAN FRESH WEIGHTS (GM.) OF THE VEGETATIVE PORTION ABOVE THE UNIFOLIATE LEAVES OF EIGHT PLANTS*

2,4-Dichlorophenoxyacetic acid (mg. per square yard)	Carrier solutions			
	Aqueous	0.5% Carbowax 1500	0.5% Carbowax 1540	0.5% Carbowax 4000
	Soybeans			
0.0 (control)	0.80	0.79	0.85	0.81
1.0	0.76	0.70	0.64	0.45
5.0	0.42	0.38	0.39	0.34
10.0	0.32	0.34	0.34	0.26
	Kidney beans			
0.0 (control)	1.20	1.22	1.16	1.19
0.5	1.05	0.96	0.98	0.87
2.5	0.72	0.28	0.23	0.31
5.0	0.43	0.15	0.15	0.18

* Minimum difference between means of soybeans for significance at the 5% level of probability is 0.12 gm., at the 1% level of probability, 0.16 gm. Minimum difference between means of kidney beans for significance at the 5% level of probability is 0.18 gm., at the 1% level of probability, 0.24 gm.

The 2,4-dichlorophenoxyacetic acid produced more highly significant inhibition of growth in kidney-bean plants when sprayed in a solution containing 0.5% of Carbowax 1500, 1540, or 4000 than when applied in a wholly aqueous spray. On the other hand, there was a striking difference in the response of soybean plants which were treated similarly. Excepting the 1-mg. applications of 2,4-dichlorophenoxyacetic acid in 0.5% Carbowax 4000 solution, soybean plants sprayed with the compound in Carbowax solutions were not inhibited in growth any more than the plants treated with wholly aqueous solutions.

Since soybean and kidney-bean plants differ markedly in the degree of leaf pubescence, it is suggested that the dissimilarity in the response of these plants to aqueous-Carbowax spray application of 2,4-dichlorophenoxyacetic acid may be attributed to this morphological difference. It appears plausible that the compound may be readily absorbed by the leaf hairs of soybeans, whereas the more glabrous leaves of kidney beans perhaps do not absorb the wholly aqueous spray so rapidly and the presence of Carbowax effects the retention of moisture on the leaf surface for an interval sufficient to permit absorption of the compound by the epidermal leaf tissues. Further study will be necessary to determine how and

in what form the substance enters the plant, before this differential response can be fully explained.

The value of Carbowax as a co-solvent for increasing the concentration of 2,4-dichlorophenoxyacetic acid in aqueous solutions has been suggested (1). ZIMMERMAN (4) has found that, in general, salts, esters, and amides are approximately equal in activity to the acids from which they are derived. This has been borne out in somewhat extensive studies, not reported here, which have indicated that, for spraying many types of broadleaf plants, the ammonium salt form of the acid is not statistically different from the acid in its effectiveness as a plant growth-regulator. Many of the salts, such as the ammonium and sodium salt, are directly soluble in water. Considering that it requires 4.5 parts by weight of Carbowax 1500 to prepare a solution containing 1 part by weight of 2,4-dichlorophenoxyacetic acid (1), the proportional amount of Carbowax required to prepare concentrations equal to that of the directly water-soluble ammonium salt is large and relatively expensive. The results of this study indicate that Carbowax offers no advantage for increasing the inhibitory action of 2,4-dichlorophenoxyacetic acid upon the soybean. Since there are many broadleaf plants that have morphological characteristics similar to the soybean, the general use of Carbowax as a co-solvent may not be justified in herbicidal sprays. It is suggested that attention be given to the use of the directly water-soluble ammonium salt or other water-soluble salt form of 2,4-dichlorophenoxyacetic acid for general herbicidal-spray purposes.

Summary

1. Soybean plants differ from kidney-bean plants in their response to aqueous-spray applications of 2,4-dichlorophenoxyacetic acid containing Carbowax.

2. The addition of any of three Carbowaxes to aqueous-spray solutions of 2,4-dichlorophenoxyacetic acid enhanced its activity in inhibiting growth of kidney-bean plants but did not aid in inducing inhibitory responses in soybean plants.

3. Because of its solubility properties, the ammonium salt of 2,4-dichlorophenoxyacetic acid is suggested instead of the acid form for use in general herbicidal sprays.

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TWO METHODS FOR THE DETERMINATION OF THE HERBICIDAL EFFECTIVENESS OF PLANT GROWTH-REGULATING SUBSTANCES IN OIL SOLUTION ON BROADLEAF PLANTS[1]

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Introduction

Oil solutions of plant growth-regulating substances applied as herbicides possess several advantages over aqueous solutions. In the first place, the oil solutions generally produce a greater inhibitory effect per unit of compound, probably because of their lower rate of evaporation and the more rapid entry of the agent into the plant tissues as a result of the ready penetration of the leaf cuticle by such solutions. Second, rainfall, if occurring shortly after exposure of the plant to the agent, severely reduces the effectiveness of aqueous, but not of oil, sprays (4). Lastly, the ready miscibility in oil of various esters of the phenoxyacetic acid series and the relative ease with which many such herbicidal agents may be dissolved in tributylphosphate and oil (1), permit high concentrations of the agent to be obtained. Such highly concentrated oil sprays have been shown to be most effective at very low volume rates of application. On the other hand, the solubility of the phenoxyacetic acids and their salts in aqueous solution is, in general, of a rather low order.

To test the effectiveness of various herbicidal agents in oil, it was first necessary to develop adequate testing methods, two of which are described below. Aside from any specificity of action which a herbicidal compound may exhibit on a particular plant species, these methods have been found satisfactory for the testing of many hundreds of compounds.

[1] Studies conducted at Camp Detrick, Frederick, Md., from September, 1944, to September, 1945, under the supervision of Dr. A. G. Norman.

Experimental

THE OIL-DROPLET TEST

This test is a modification of the water-droplet test of MITCHELL and HAMNER (2). Kidney beans were used as test plants, and the herbicidal compounds were applied when the primary leaves had expanded and the second internode was 1 inch in length. The compounds were put into solution and tested in the following manner: 50 mg. were dissolved in 0.2 or 0.4 ml. of tributylphosphate, and a sufficient amount of no. 2 fuel oil (kerosene base) was added to bring the total volume to 10.0 ml. If 50 mg. of the compound were not soluble in 0.4 ml. of tributylphosphate, the compound was arbitrarily considered insoluble. After a thorough shaking, the solution was allowed to stand for a day to determine whether precipitation occurred. Of this stock solution, 1.0 ml. was diluted to 10 ml. with fuel oil to give a 500 p.p.m. solution. Six kidney-bean plants were then treated by placing a single drop of solution (0.01 ml.), containing 5γ of agent, on the base of the blade of one of the two primary leaves of each plant. A $\frac{1}{4}$-ml. tuberculin syringe was used to give uniform droplet size. The plants were allowed to grow for 10 days, after which all growth above the primary leaves was removed and fresh weights were obtained. Oil and tributylphosphate-oil controls were used with each group of

compounds to be tested. The data were not statistically analyzed.

The oil-spray test

This was designed to test for small differences in effectiveness, based on a low amount of agent and small volume of solution applied at sublethal levels. Soybean plants were used because of their uniformity of growth and were exposed when the second trifoliate leaf was fully expanded. Four pots of three plants each were sprayed. Data from the results were then statistically analyzed.

The solutions of compounds to be tested were obtained by adding 1 ml. of the 0.5% stock solution (see "Oil-Droplet Test," above) to 1.5 ml. of fuel oil. This, when sprayed in a chamber with a base area of $\frac{1}{2}$ square yard, gave an application of 10 mg. of agent in a 5-ml. volume per square yard. The solutions were applied with an atomizer attached to a laboratory air jet at approximately 15 pounds pressure. The plants were then allowed to grow for 21 days under normal greenhouse conditions, at the end of which time all growth above the second trifoliate leaves was removed and fresh weights were taken. Oil and tributylphosphate-oil controls, using the same volume sprays, were used with each group of compounds tested.

Discussion

These two tests, both employing growth-inhibition as a criterion of effect, provide ready means for evaluating the herbicidal effectiveness of many compounds in oil solution. It was necessary to supplement the oil-droplet test, which is principally a preliminary screening procedure for the determination of the better herbicidal compounds, with the oil-spray test, because of variable data obtained from the former. This variability depends primarily on the angle of attachment of the primary leaf on which the oil drop is placed, the angle governing the amount of oil, and hence the amount of agent, which will spread down the petiole and reach the base of the second internode. On the other hand, the water-droplet test of Mitchell and Hamner (2) and the corn-germination test (3), judging from the results obtained in these laboratories, appear to be thoroughly satisfactory for accurately evaluating the action of herbicidal agents in aqueous solution. The aqueous insolubility of many compounds, however, and the practicability of using herbicidal oil sprays require the use of some method of testing compounds in oil solutions. Despite the variability of the oil-droplet test, it is sufficeintly accurate to be of value for preliminary screening, when one considers the ease and rapidity with which many growth-regulating substances may be tested. The definitely poorer compounds can be discarded and the better ones further screened by the oil-spray test. A comparison of some of the better phenoxyacetic acid derivatives tested by these two methods is given in table 1. It will be apparent from the data that, in oil solutions, derivatives of 2,4,5-trichlorophenoxyacetic acid produce a greater inhibitory effect in general than either 4-chloro derivatives or the 2,4-dichlorophenoxyacetic acid.

Summary

Two tests are described which permit an evaluation of the herbicidal effectiveness on broadleaf plants of growth-regulating substances dissolved either in oil alone or in tributylphosphate and oil. The first, the oil-droplet test, is less reliable than the oil-spray test, but its value lies in the ease and rapidity with which a large number of substances may

be tested. The second test, based on low rates and volumes of application, is designed to test for small differences, and it has proved very accurate. Both tests use growth inhibition as a criterion of herbicidal effectiveness.

TABLE 1

COMPARISON OF THE FRESH WEIGHTS (EXPRESSED IN % OF CONTROL) OF PLANTS TREATED WITH VARIOUS HERBICIDAL COMPOUNDS BY THE OIL-DROPLET AND OIL-SPRAY TESTS

Compound	Oil-droplet test	Oil-spray test
Untreated control	100	100
4-chlorophenoxyacetic acid	48	40
4-chlorophenoxyacetyl chloride	54	52
4-chlorophenoxyacetic phenylhydrazide	26	23
Beta-chloroethyl 4-chlorophenoxyacetate	12	40
2-methyl, 4-chlorophenoxyacetic acid	70	45
2,4-dichlorophenoxyacetic acid	41	38
2,4-dichlorophenoxyacetic acid*	95	70
Beta-bromoethyl 2,4-dichlorophenoxyacetate	9	48
Beta-chloroethyl 2,4-dichlorophenoxyacetate	20	51
Isoamyl 2,4-dichlorophenoxyacetate	45	56
Trichloroethyl 2,4-dibromophenoxyacetate	42	55
Ethyl 2,4-dichlorophenoxyacetate	53	44
Beta-bromethyl 2,4-dichlorophenoxyacetate	17	51
Butyl 2,4-dichlorophenoxyacetate	35	46
2,4,5-trichlorophenoxyacetic acid	22	14
2,4,5-trichlorophenoxyacetyl chloride	34	20
2,4,5-trichlorophenoxyacetic 2′,4′-dimethylanilide	11	13
2,4,5-trichlorophenoxyacetic 2′,4′,6′-trichloroanilide	16	18
2,4,5-trichlorophenoxyacetic meta-chloroanilide	13	18
2,4,5-trichlorophenoxyacetic para-anisidide	50	27
2,4,5-trichlorophenoxyacetic beta-naphthanilide	16	9
2,4,5-trichlorophenoxyacetic para-bromanilide	14	42
2,4,5-trichlorophenoxyacetic di(beta-hydroxy-ethyl)amide	27	22

* Dissolved in water.

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EFFECT OF PLANT GROWTH-REGULATORS IN RELATION TO STAGES OF DEVELOPMENT OF CERTAIN DICOTYLEDONOUS PLANTS[1]

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Introduction

Before an effective program of weed control in dicotyledonous crops can be developed, the responses of the various crop species to different rates and methods of application of chemical herbicides must be known at all stages of development. In the absence of selective herbicides which will kill one species of broadleaf plant but not others, a differential susceptibility between crop and weed, depending upon stage of development, may possibly provide an avenue of approach to weed control.

Preliminary greenhouse studies showed that, in general, the responsiveness of a plant to herbicidal applications of plant growth-regulators decreased with increasing age when fresh or dry weight of the aerial portion of the plant was taken as the criterion of effectiveness of the treatment. However, only in such crops as lettuce, cabbage, broccoli, cauliflower, etc., are the aerial portions used for food. In other crops either the fruit, the seed, or some underground portion of the plant is used for edible purposes. It was the purpose of this investigation to determine at which stage of development each of a variety of crops is responsive to such chemical herbicides and to compare their responsiveness with the responsiveness of such vegetative crops as cabbage.

[1] Work conducted at: Camp Detrick, Frederick, Md., from June, 1944, to September, 1945, under the supervision of Dr. A. G. Norman.

Methods

Applications of the ammonium salt of 2,4-dichlorophenoxyacetic acid in aqueous solution were made by spraying. Field spraying was done on relatively windless days, with the plots to be treated inclosed by canvas screens 4 feet high to prevent drifting of the spray. A DeVilbiss paint sprayer, with no. 92 detachable nozzle, was used at 10 pounds of pressure, the air supply being derived from a portable cylinder. With this apparatus and pressure, the spray consisted of relatively large droplets, which settled quickly. A volume of 10.0 ml. of spray per square yard of treated area was used throughout the experiments. Adequate spray coverage could be readily achieved at this volume.

The ammonium salt of 2,4-dichlorophenoxyacetic acid was employed instead of the acid itself, since concentrations of the salt up to 2.9% can be obtained in water. When higher concentrations were required, sufficient diethylamine was added to dissolve the acid.

The usual agronomic practices were carried out in growing the plant materials.

Experimental results

Cabbage

Single plots of four 10-foot rows of Jersey Wakefield cabbage were treated at three stages of development and at rates of 0.03 and 0.3 gm. of ammonium

2,4-dichlorophenoxyacetate per square yard. This corresponds closely to rates of 0.3 and 3.0 pounds of the compound per acre. Fresh weights of heads were taken approximately 1 month after the last spray application (table 1).

TABLE 1

FRESH WEIGHT (POUNDS) OF CABBAGE HEADS PER PLOT TREATED WITH AQUEOUS-SPRAY APPLICATIONS OF AMMONIUM 2,4-DICHLOROPHENOXYACETATE

RATE (GM. PER SQUARE YARD)	STAGE WHEN TREATED		
	6–8 leaves	Beginning to head	Heads 4 inches in diameter
0.03	0.0	0.0	15.5
0.30	0.0	0.0	13.0
Control (untreated)	17.0	.....	.....

TABLE 2

MEAN AIR-DRY WEIGHT (GRAMS) OF THRESHED SOYBEANS PER PLOT TREATED WITH AQUEOUS-SPRAY APPLICATIONS OF AMMONIUM 2,4-DICHLOROPHENOXYACETATE AT A RATE OF 0.5 POUND PER ACRE. FOUR REPLICATES*

Stage when treated	Average weight of beans per plot
3 inches	135.5
6–8 inches high	145.5
Early flowering	72.0
Early pod	22.8
Control	161.7

* Minimum difference between means for significance at 5% level of probability is 69.3 gm.; at 1% level it is 95.8 gm.

Cabbage was more susceptible to the herbicide in the early stages of growth than it was after the heads had begun to form. At the two earlier stages even the lower rate of application proved rapidly lethal. A relatively mature stage was quite resistant to this herbicide, little inhibition of growth being achieved at the rates used when the heads were over 3 inches in diameter. This trend of responses was similar to that found in greenhouse spray experiments on a variety of plants, when inhibition of the aerial portion of the plant was used as a criterion of the effectiveness of the herbicide.

SOYBEANS

Soybeans, variety Chief, were drilled on May 2, 1945, in 6 × 9-foot plots and treated at four stages of development with a single rate of 0.5 pound of ammonium 2,4-dichlorophenoxyacetate per acre. The pods were allowed to mature, and the plants were harvested on September 28, 1945. The weights of air-dried threshed beans were recorded (table 2).

The immediate effect of the spray treatments on the plants, particularly the two youngest stages, was striking. All young parts showed considerable stem curvature and leaf epinasty, followed later by cortical splitting. This latter phenomenon was due undoubtedly to cell proliferation and root formation in the phloem-cambial region (3). Older plants, treated at the early pod stage, had attained maximum vegetative growth, and the initial effects were less striking.

When the weight of threshable beans was used as a criterion, the later applications were found to be most effective (table 2), which is the converse of the vegetative responses. The younger stages, while severely retarded and considerably distorted by the spray applications, nonetheless showed a remarkable capacity for recovery, eventually producing a normal yield. Older plants, on the other hand, produced significantly reduced yields, while showing little visible effect following treatment. These results parallel those of GRIGSBY (1), who showed that floral formation and seed set in ragweed

could be severely curtailed by applications of 2,4-dichlorophenoxyacetic acid. Undoubtedly, the developing floral and ovarial structures were as severely affected by this herbicide as was the young vegetative growth. The latter could, however, overcome the initial effects by further vegetative growth, but the former could not, since inhibitory influences in the floral structure generally resulted in sterility.

To determine whether the method of application affects the relative susceptibility at various growth stages, soil treatments were also tried. Soybeans were treated at four stages at rates of 3, 10, and 20 pounds of 2,4-dichlorophenoxyacetic acid per acre. The growth-regulator was impregnated in 10-mesh sawdust or processed as granular material, with Fuller's earth as a diluent to facilitate ease of distribution. The treatment materials were uniformly applied by hand to the soil, and care was exercised to prevent them from coming into direct contact with vegetative portions of the plants. All treatments were made in quadruplicate, with each plot consisting of three rows of drilled soybeans 16 feet long. The beans were harvested on September 28, 1945 (table 3).

In addition to the observation that soil applications were relatively inefficient as compared to spray treatments, it was also apparent that the effect of stage of development was not comparable. Only when the herbicide was applied at planting time was a significant reduction in yield obtained. Established plants were relatively unaffected. The extreme sensitivity of plants, when treatments were made at planting time, was not unexpected, as it has been shown that germinating seeds are very susceptible to small applications of herbicidal growth-regulators (**2, 4**).

TABLE 3

AVERAGE WEIGHT IN GRAMS PER PLOT (48 ROW FEET) OF THREASHED SOYBEANS TREATED AT FOUR STAGES OF DEVELOPMENT WITH THREE RATES OF 2,4-DICHLOROPHENOXYACETIC ACID IN TWO TYPES OF CARRIERS. FOUR REPLICATES*

POUNDS PER ACRE	STAGE WHEN TREATED			
	Planting time	6–8 inches high	Early flowering	Early pod
Sawdust:				
3	385.5	499.2	392.5	391.5
10	170.8	541.0	441.0	388.5
20	93.8	456.0	336.2	449.8
Granular material:				
3	560.8	425.5	489.2	459.2
10	240.2	430.8	379.8	410.0
20	189.0	400.0	333.5	421.2
Control (untreated)	439.8	493.5	462.8	501.2

* Minimum difference between means for significance at the 5% level of probability is 139.0 gm.; at 1% level it is 184.1 gm.

TOMATO

In greenhouse trials young tomato plants appeared to be particularly sensitive to aqueous sprays of halogenated derivatives of the phenoxyacetic acid series at low concentrations. The vegetative growth was readily inhibited, and the fruits, when produced, were small and misshapen. In the field, however, the tomato was somewhat similar to the soybean in its response to aqueous sprays of ammonium 2,4-dichlorophenoxyacetate (table 4).

Tomato plants, in single 12 × 12-foot plots, were treated at three stages of development, and at rates of 0.1 and 1.0 pounds per acre of ammonium 2,4-dichlorophenoxyacetate. The fruits were harvested approximately 1 month after

the final treatments and at a time when the majority of all fruits were well colored.

As with soybeans, young tomato plants, unless killed, showed a considerable capacity for vegetative recovery, with only a slightly reduced yield. The fruits appeared normal. When they were sprayed in the early flowering stage, not only were the blossoms present at the time of application destroyed, but all subsequent floral development was curtailed, and no fruit was set. When applied at the early fruiting stage, a considerable reduction in yield was achieved at the 1 pound per acre rate, with the fruits formed somewhat misshapen and frequently seedless. The 0.1 pound per acre rate had but little effect at this stage, and the fruits were normal. In this respect the tomato differed from the soybean in its response to ammonium 2,4-dichlorophenoxyacetate, since in the soybean the early pod (fruiting) stage was the most sensitive of all stages in respect to fruit set (table 2).

TABLE 4

FRESH WEIGHT IN GRAMS OF TOMATOES TREATED AT THREE STAGES OF DEVELOPMENT WITH AMMONIUM 2,4-DICHLOROPHENOXYACETATE AT RATES OF 0.1 AND 1.0 POUNDS PER ACRE

RATE (POUNDS PER ACRE)	STAGE WHEN TREATED		
	8–12 inches high	Early flowering	Early fruiting
0.1	3268	0	4315
1.0	0*	0	1415
Control	6525	.....	.....

* Plants killed.

TABLE 5

AVERAGE FRESH WEIGHT IN GRAMS OF MATURE SWEET-POTATO TUBERS FROM 9 × 9-FOOT PLOTS TREATED WITH TWO RATES OF AMMONIUM 2,4-DICHLOROPHENOXYACETATE AT TWO STAGES OF DEVELOPMENT. FOUR REPLICATES

POUNDS PER ACRE	STAGE WHEN TREATED	
	Early runner	Vines covering ground
0.25	0	733
1.0	0	69
Control (untreated)	5093	.....

SWEET POTATOES AND SUGAR BEETS

Puerto Rico sweet potatoes and Wanzleben sugar beets were treated to determine the effect of aqueous sprays of ammonium 2,4-dichlorophenoxyacetate on yield of root crops. No yield data were obtained from the plantings of sugar beets because treatments of as little as 0.5 pounds per acre at two stages of development—5–7 inches in height and 12–14 inches in height with roots 4–6 inches in length and thumb-size in diameter—effectively killed all plants.

Sweet potatoes were planted on May 17, 1945, as well-rooted slips spaced 18 inches apart in 3-foot rows. Some plants were treated on July 24, when they were in the early runner stage, and others on August 14, when the vines had completely covered the ground. Plants were sprayed with an aqueous solution of ammonium 2,4-dichlorophenoxyacetate at rates of 0.25 and 1.0 pounds per acre. On October 3, 1945, the potatoes were dug and weighed. The data of table 5 indicate that susceptibility of sweet potatoes to the growth-regulator decreased with increasing age and size of plant. The sweet potato was, however, affected by very low dosages of the compound, being one of the most readily killed of the crop plants studied.

Discussion

The data obtained from herbicidal-spray trials applied at various stages of development showed that the selection of the most susceptible stage of any crop depends on the criterion of effect used. With vegetative growth, either aerial or root, employed as the criterion, the earliest stages of growth, in general, showed the greatest inhibitory response. If fruit set was used for the evaluation of response, the early flowering or early pod stage was the most readily affected period of growth. These differences, however, are readily reconcilable when one considers that in each instance it is immature structures—i.e., immature vegetative growth—or floral parts which respond to the treatments, thus causing inhibitory effects which are reflected in the final yields.

In any weed-control program it will be necessary, therefore, to spray at the proper time if a differential weed-killing is to be achieved, and no one time or rate will be suitable for all crops. Sufficient data are not available to state conclusively which developmental stage is the most susceptible, although it would appear from the soybean spray trials that an application which will effectively prevent seed set when applied at the flowering stage may not kill even the youngest plants.

It is probable that quantities of herbicidal compounds which cause no damage to certain crop plants would be lethal doses for many young or seedling weeds. The experiments in this paper indicate roughly the levels of 2,4-dichlorophenoxyacetic acid that will not injure certain crop plants. Experiments concerned with determination of minimum rates of herbicide necessary to kill weed species at various stages of development would be of value.

Summary and conclusions

1. Five species of field crops (cabbage, soybean, tomato, sweet potato, and sugar beet) at various developmental stages were treated with aqueous-spray applications of ammonium 2,4-dichlorophenoxyacetate. Soybeans were also treated with soil applications of 2,4-dichlorophenoxyacetic acid.

2. Cabbage at three stages of growth (six to eight leaves, beginning to head, and heads 4 inches in diameter) were sprayed with this growth-regulator at rates of 0.3 or 3.0 pounds per acre. The two younger stages were killed by the compound, but the oldest plants were little inhibited in growth.

3. Soybeans at four stages of development were sprayed with the herbicide at a rate of 0.5 pounds per acre. The two younger stages (3 inches and 6–8 inches in height) were for a time severely retarded in growth and distorted by the treatment, but these plants eventually recovered and produced a normal yield of beans. The two older stages (early flowering and early pod) showed less visible response immediately following treatment, but subsequent yields of beans were significantly reduced. These data indicate that developing floral and ovarial structures are as severely affected by the herbicide as is young vegetative growth.

4. Soybeans at planting time and at three developmental stages also received soil applications of 2,4-dichlorophenoxyacetic acid at rates of 3, 10, or 20 pounds per acre. Only when the herbicide was applied at planting time was there a significant reduction in the yield of beans obtained. The lowest application rate caused no reduction in yield. Established plants were relatively unaffected.

5. Tomatoes at three developmental

stages received spray treatments of the herbicide at rates of 0.1 or 1.0 pounds per acre. Young plants (8–12 inches high), unless killed, showed a considerable capacity for vegetative recovery, with only a slightly reduced fruit yield. When plants were sprayed at the early flowering stage, no fruit was set. When treatment was made at the early fruiting stage, there was a reduction in yield, and the fruits were somewhat misshapen and frequently seedless.

6. Sweet potatoes and sugar beets were treated to determine the effect of the herbicidal spray on yield of root crops. No yield data were obtained for sugar beets because treatment with 0.5 pound per acre killed plants at the two stages of development studied (5–7 inches in height and 12–14 inches in height, with roots 4–6 inches in length and thumb-size in diameter). Sweet potatoes at early runner stage or when vines completely covered the ground were treated at rates of 0.25 or 1.0 pounds per acre. Plants at the younger stage were killed by both rates of the compound and root yield of older plants was greatly inhibited, even at the lower treatment level. The susceptibility of the sweet potato to the growth-regulator decreased with increasing age and size of plant.

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EFFECTS OF CERTAIN GROWTH-REGULATING COMPOUNDS ON IRISH POTATOES[1]

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Introduction

Herbicidal agents selective in character might find widespread use in agriculture if they removed undesirable weeds without causing concurrent injury to a growing crop. It has been demonstrated that some of the substituted phenoxyacetic acids may be so employed in the control of broadleaf weeds in cereal corps (2). A herbicide which kills or inhibits plant growth indiscriminately, however, would be of little use in eliminating weeds from economic crops, since the crop would suffer as well.

During an extensive study of the effects of herbicidal agents on many broadleaf crops, it was noticed that, while 2,4,5-trichlorophenoxyacetic acid had a deleterious effect on the Irish potato, such agents as 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid produced only little inhibition of either vegetative growth or tuber yield.

[1] Studies conducted at Camp Detrick, Frederick, Md., from January, 1945, to September, 1945, under the supervision of Dr. A. G. Norman.

Further studies were then carried out, and it is the purpose of this paper to present the results obtained.

Experiment 1

This experiment was conducted to test the effect upon the potato of several plant growth-regulating substances containing the phenoxyacetic grouping and certain others unrelated in structure. The compounds were applied in aqueous sprays to potted greenhouse Irish potato plants about 1 foot in height. Each compound was applied in quadruplicate at the rate of 0.1 gm. per square yard in 50 ml. of aqueous solution. The applications were made in a spray chamber 0.5 square yard in area and 4 feet deep, with high-pressure Precision sprayers of 200-ml. capacity.

One month after treatment, plant height measurements were made, and 2 months after treatment, fresh weights of all tubers produced per half-gallon glazed pot were taken.

It is evident from the data of table 1 that ammonium 2,4,5-trichlorophenoxyacetate significantly reduced the yield of tubers and also stunted the vegetative growth. One month after spray applications, the plants treated with this compound were stunted in growth and showed swelling of the pulvini and thickening of the stem immediately adjacent to the petiole. Potato plants sprayed with ammonium 2,4-dichlorophenoxyacetate and 2-bromo-3-nitro-benzoic acid developed epinastic responses soon after treatment, but the plants appeared to have completely recovered within a few days.

The tubers from the plants treated with ammonium 2,4,5,-trichlorophenoxyacetate were markedly reduced in size and had a warty, scab-like appearance (fig. 1), whereas none of the other compounds tested in this experiment caused evident injury to the tubers. Under the conditions of the experiment, ammonium 2-methyl-4-chlorophenoxyacetate reduced the yield of tubers significantly, but the tubers were normal in appearance.

It was noticed at the time of harvest that those plants that had been treated with 2,4-dichlorophenoxyacetic acid had set a considerable number of seed balls. None of the control plants or those treated with other compounds set any fruit. Although no attempt was made to test the seed for viability (they were harvested before maturity), it appears that this compound might be used to cause the setting of fruits after fertilization on such crops as potatoes, where premature abscission often limits or prevents the maturation of fruit.

TABLE 1

Effect of certain growth-regulators on Irish potatoes. Applications in aqueous sprays at rate of 0.1 gm. per square yard in 50 ml. of solution. Figures represent mean fresh weight of tubers in grams and mean height of plants in inches*

Treatment	Mean fresh-weight tubers†	Mean plant height‡
Untreated	64.4	31.6
NH_4 2,4,5-trichlorophenoxyacetate	9.6	16.2
NH_4 2-methyl-4-chlorophenoxyacetate	45.0	29.5
NH_4 2,4-dichlorophenoxyacetate	56.7	26.5
NH_4 para-chlorophenoxyacetate	62.6	26.6
NH_4 2-bromo-3-nitrobenzoate	57.6	26.2
NH_4 2,3,5-triiodobenzoate	70.3	27.8
NH_4 2-methyl-4,6-dichlorophenoxyacetate	66.9	28.5
Isopropyl phenylcarbamate	69.1	27.6
2,5-Dichlorophenoxyacetic acid	58.0	29.2
2,4-Dibromophenoxyacetic acid	61.8	28.4

* Minimum difference for significance between means at the 5% level of probability is 11.2 gm. (tubers) and 12.0 inches (height).

† Taken 2 months after treatment.

‡ Taken 1 month after treatment.

EXPERIMENT 2

In Experiment 1 the greater effectiveness of ammonium 2,4,5-trichlorophenoxyacetate over ammonium 2,4-dichlorophenoxyacetate in causing inhibition of vegetative growth and tuber yield suggested that the chlorinated 5 position in the benzene ring might be responsible for the degree of specificity exhibited by this compound on the Irish potato. This experiment was designed to test this hypothesis, using, among others, some compounds with the 3 or 5 position chlorinated.

Greenhouse-grown Irish potato plants in 6-inch clay pots were arranged into treatment groups of four plants each when the plants were 3–8 inches in height. Care was exercised to have good uniformity between treatment groups, but there was considerable variation in size within treatment groups. Four plants were harvested at the time of treatment, and no tubers had formed at that time. Many of the compounds were difficultly water-soluble and accordingly were incorporated into oil, using tributylphosphate as a co-solvent (1). A low-volume spray of 5 ml. per square yard was applied, with each treatment solution containing 4 per cent tributylphosphate and 100 mg. of the compound. This treatment is roughly equivalent to a rate of 1 pound of acid per acre. The plants were sprayed in quadruplicate in a spray chamber 0.5 square yard in area and 4 feet deep, with a De Vilbiss atomizer operated under approximately 15 pounds air pressure. Fresh weights were taken of the tubers and of the vegetative portion of the plants 54 days after treatment.

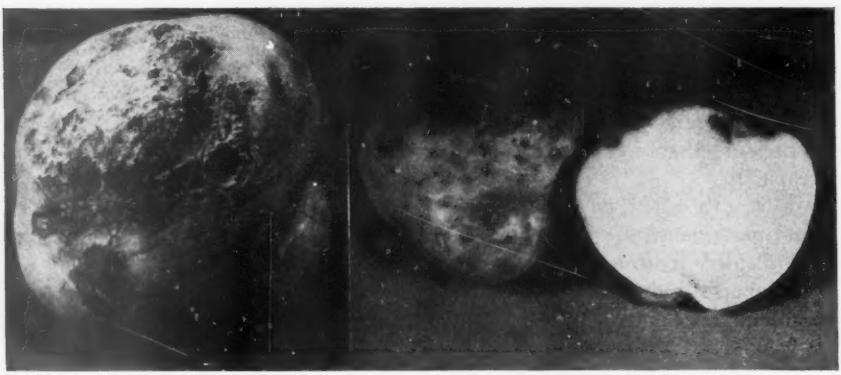

FIG. 1.—Typical injury to potato tubers caused by 2,4,5-trichlorophenoxyacetic acid applied by spray either to vegetative portions of the plant or to the soil. Injury is largely superficial.

Only the compounds which possessed the 2,4,5-chlorophenoxy configuration—2,4,5-trichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetylchloride—caused significant decreases in the yield of potato tubers (table 2). One compound—3,5-dichlorophenoxyacetic acid—reduced the weight of vegetative growth but did not reduce the weight of tubers.

The significance of the 2,4,5-trichlorophenoxy configuration in causing injury to the potato was further borne out in another greenhouse comparison not re-

ported here, in which twenty substituted phenoxyacetic acids were tested on potatoes. Only the compounds possessing the 2,4,5-trichlorophenoxy configuration—namely, 2,4,5-trichlorophenoxyacetic anilide, 2,4,5-trichlorophenoxyacetic ortho-nitroanilide, 2,4,5-trichlorophenoxyacetic meta-nitroanilide, and 2,4,5-trichlorophenoxyacetonitrite—caused marked stunting of potato plants and injury to tubers. The injury was similar to that produced by 2,4,5-trichlorophenoxyacetic acid. No yield data were taken because of the large plant variability resulting from high summer temperatures.

It is evident, however, from the data of table 2 that little significance can be attributed to the chlorinated 5 position of the benzene ring in so far as phytocidal action on the potato is concerned.

Experiment 3

Extensive testing of 2,4-dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid has shown that they produce inhibition and death of many broadleaf plants if applied at relatively low rates. In the present study, emphasis was directed toward the actual response of field-grown potatoes to treatment with these compounds. Both aqueous- and oil-spray solutions of the compounds were applied, since comprehensive greenhouse studies indicated that the response of plants to growth-regulators might differ when the latter were applied in oil sprays instead of in aqueous sprays.

Irish Cobbler potatoes were planted April 9, 1945, in Frankstown silt loam in rows 3 feet apart, with cut "seed" pieces spaced at 12-inch intervals. Prior to planting, an application of 1000 pounds per acre of a 4-10-5 fertilizer was broadcast and disked into the soil. When the plants were in the early bud stage (8–10 inches in height), aqueous and tributylphosphate-oil sprays of the three compounds were applied in quadruplicate at the rate of 0.1 gm. per square yard (about 1 pound per acre) in a spray volume of 10 ml. per square yard. The aqueous sprays were prepared by dissolving 8.0 gm. of each of the acids in 800 ml. of water, to which had been added 5 ml. of NH_4OH and 3 ml. of diethylamine. The oil sprays were prepared by dissolving 8.0 gm. of each of the acids in 16 ml. of tributylphosphate and diluting to 800 ml. with no. 2 fuel oil. Each spray solution was applied uniformly to plots 12 × 12 feet with a modified De Vilbiss type CV paint-spray apparatus with 10 pounds constant air pressure from a pressure cylinder. The plots were protected from the effect of wind during the spray operation with canvas-covered

TABLE 2

Effect of certain growth-regulators on Irish potatoes. Application in oil sprays at rate of 0.1 gm. per square yard in 5 ml. of solution. Figures represent mean fresh weight in grams taken 54 days after treatment*

Treatment	Mean fresh-weight tubers	Mean plant weight
Control (untreated)	35.4	57.9
4% tributylphosphate-oil control	37.0	59.9
2,4,5-trichlorophenoxyacetic acid	5.4†	28.2†
2,5-dimethylphenoxyacetic acid	40.6	55.4
3-chlorophenoxyacetic acid	38.2	59.4
2,5-dichlorophenoxyacetic acid	21.8	64.6
3,5-dichlorophenoxyacetic acid	50.2	43.0†
3,4-dichlorophenoxyacetic acid	18.5	58.5
2,4,5-trichlorophenoxyacetyl chloride	13.2‡	36.1†
2,3,5-trichlorobenzoic acid	29.1	63.5
Butyl 2,4-dichlorophenoxyacetate	45.7	55.7

* Minimum difference for significance between means at 5% level of probabiliiy is 22.8 gm. (tubers) and 5.7 (vegetative plant).

† Significantly lower in yield than untreated at 99:1 odds.

‡ Significantly lower in yield than untreated at 19:1 odds.

shields 4 feet high. The plants were harvested, and tubers were weighed from each plot on July 27 after all vines were dead.

TABLE 3

EFFECT OF THREE GROWTH-REGULATORS ON IRISH POTATOES. APPLICATIONS IN AQUEOUS AND OIL SPRAYS AT RATE OF 0.1 GM. PER SQUARE YARD IN 10 ML. OF SOLUTION. FIGURES ARE MEAN YIELDS PER PLOT (16 SQUARE YARDS) OF ALL TUBERS 57 DAYS AFTER SPRAY APPLICATION*

TREATMENT	MEAN YIELD PER PLOT (IN POUNDS)	
	Aqueous spray	Oil spray
2,4-dichlorophenoxyacetic acid	10.8	5.0
2-methyl 4-chlorophenoxyacetic acid	15.2	7.6
2,4,5-trichlorophenoxyacetic acid	14.2	2.7
Control (untreated)	14.8	

* Minimum difference for significance between means at the 5% level of probability is 1.9 pounds.

It is evident from the data of table 3 that the oil sprays of the compounds caused a much greater reduction in yield of tubers than did the aqueous sprays. Although 2,4,5-trichlorophenoxyacetic acid did not reduce the yield of tubers substantially when applied in an aqueous spray, the quality of tubers produced was very poor (fig. 2). On the other hand, 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid applied to potato plants in either aqueous or oil sprays did not affect the quality of the tubers, but both reduced the yield of tubers significantly when applied in the oil spray.

EXPERIMENT 4

In view of the apparent specific action of 2,4,5-trichlorophenoxyacetic acid on the Irish potato when applied to the aerial portion of the plant, a preliminary study was made to determine whether the compound acted similarly when applied as a soil contaminant. Uniform

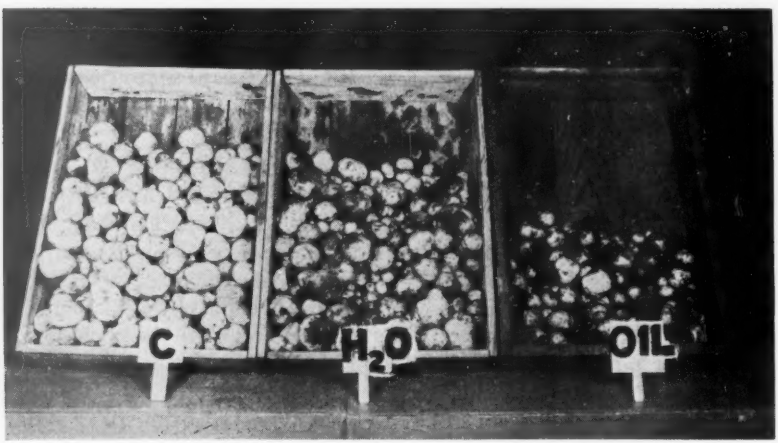

FIG. 2.—Effect upon potato tubers of 2,4,5-trichlorophenoxyacetic acid when applied to field-grown potatoes in water and oil sprays. The compound was applied at an early bud stage at the rate of 1 pound per acre in a volume of spray of 10 ml. per square yard. Greater reduction in yield and corky epidermal injury with oil spray.

potato plants, grown in 6-inch clay pots, were treated in duplicate when about 16–18 inches in height. The compounds 2,4-dichlorophenoxyacetic acid, ammonium 2,4,5-trichlorophenoxyacetate, and a 1:1 mixture of the two compounds were applied to the soil in 100 ml. of water at rates of 8, 24, and 40 mg. per pot, or approximately 4, 12, and 20 pounds per acre, respectively. Care was exercised to prevent the solutions from coming in direct contact with vegetative portions of the plants. All pots were uniformly surface-watered throughout the course of the experiment, and care was exercised to prevent leaching. Fresh weights were taken of the tubers 42 days after treatment.

Five hours after application all the treated plants showed bending of the stems and epinasty of the petioles. The degree of bending was positively correlated with the rate of application. Two days after treatment there was practically complete recovery from the effects produced by the 8 mg. per pot application of 2,4-dichlorophenoxyacetic acid, but plants in all other treatments retained some stem and petiole curvature.

One week after treatment, swelling of the pulvini was evident on all plants receiving ammonium 2,4,5-trichlorophenoxyacetate, either alone or in combination with 2,4-dichlorophenoxyacetic acid. A week later the leaves of many plants, which had showed swelling of the pulvini earlier, were broken off at the base of the petiole because of the brittle condition of the latter, probably resulting from the formation of callus-type growth with concurrent weakening of mechanical tissues. None of the rates of treatment with 2,4-dichlorophenoxyacetic acid caused apparent injury to the aerial portion of the plants 3 weeks after treatment.

A low yield (table 4) of tubers was produced by the plants treated with ammonium 2,4,5-trichlorophenoxyacetate or a 1:1 mixture of that compound and 2,4-dichlorophenoxyacetic acid, and the tubers were warty and small. Figure 1 shows typical injury. The 2,4-dichlorophenoxyacetic acid did not produce any apparent injury to the tubers.

Discussion

It is apparent from the experimental data presented that 2,4,5-trichlorophenoxyacetic acid is extremely injurious to Irish potato tubers even at low rates and volumes of application in either oil or aqueous solution. Oil solutions of 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid are likewise injurious, in that they cause some reduction in tuber yield, although, unlike 2,4,5-trichlorophenoxyacetic acid, they do not affect the marketable or edible quality of the tubers. Aqueous solutions

TABLE 4

EFFECT UPON IRISH POTATOES OF GROWTH-REGULATORS APPLIED TO SOIL. APPLICATIONS TO SOIL IN 100-ML. AQUEOUS SOLUTION PER 6-INCH CLAY POT. FIGURES REPRESENT PER CENT OF TUBER YIELD PRODUCED BY UNTREATED PLANTS. YIELDS TAKEN 42 DAYS AFTER TREATMENT. TWO PLANTS PER TREATMENT

COMPOUND APPLIED	TREATMENT RATE PER POT		
	8 mg.	24 mg.	40 mg.
Untreated	100.0	100.0	100.0
2,4-dichlorophenoxyacetic acid	104.8	83.0	71.0
2,4-dichlorophenoxyacetic acid and NH_4 2,4,5-trichlorophenoxyacetate*	73.1	62.1	0.0
NH_4 2,4,5-trichlorophenoxyacetate	52.1	22.3	0.6

* A 1:1 mixture of these compounds was applied.

of 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid, however, have no effect on either top growth or tuber yield when applied at rates and volumes of application which would be quite effective in inhibiting or killing the usual broadleaf weeds. None of the agents tested, in either oil or aqueous solution, had any marked effect on grasses.

The selective resistance of the Irish potato to 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid and the effectiveness of these chemicals in controlling the usual broadleaf weeds suggest that an aqueous herbicidal spray of either of these two agents, perhaps applied in conjunction with a fungicide or insecticide, can be effectively used as a weed-control measure.

Summary

1. The substance 2,4,5-trichlorophenoxyacetic acid and other compounds with the 2,4,5-trichlorophenoxy- configuration have been shown to have a decided herbicidal action on the Irish potato, while other phenoxyacetic acid derivatives applied in sprays at comparable rates produce markedly less and different effects.

2 The compound 2,4,5-trichlorophenoxyacetic acid, when applied to vegetative portions of Irish potato plants in aqueous or oil sprays, or to the soil, causes pronounced stunting and distortion of vegetative growth, swelling of the pulvini, and marked reduction in yield and quality of tubers.

3. The chlorinated 5 position of substituted phenoxyacetic acids does not appear to have any significance as far as the phytocidal action of such compounds on the potato is concerned, whereas the 2,4,5-trichloro- configuration appears to be significant.

4. The use of substituted phenoxyacetic acids, such as 2-methyl-4-chlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid, may be of value for the selective control of weeds in potato fields. Further testing of such compounds for herbicidal use against weeds in potato fields is suggested.

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SOME EFFECTS OF PLANT GROWTH-REGULATORS ON SEED GERMINATION AND SEEDLING DEVELOPMENT[1]

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Introduction

Several recent reports in the literature have established that certain halogenated phenoxyacetic acid compounds and isopropyl-N-phenylcarbamate have herbicidal properties upon established plants (1, 2, 3, 4). The investigations reported in this paper were undertaken to determine the effects of these compounds upon the germination and early seedling stages of plants.

Experimentation and results

STUDIES IN MOIST CHAMBERS

Preliminary studies of the effect of 2,4-dichlorophenoxyacetic acid upon germination and seedling development of twenty-two cereal and broadleaf crops were made in glass chambers about 10 inches in diameter. Twenty-five seeds were placed upon four layers of filter paper, 100 ml. of solutions, which varied in concentration from 0.01 to 100 parts per million, were added, and the glass dish was placed in a darkroom maintained at 25°–27° C. Observations were made daily for 4 days. Tests were made with kidney beans, navy beans, soybeans, peas, cowpeas, Korean lespedeza, turnips, radishes, rutabagas, sugar beets, sunflowers, cotton, muskmelons, cucumbers, buckwheat, maize, wheat, oats, rice, sorghum, barley, and millet.

The general effect of the 2,4-dichlorophenoxyacetic acid was to retard the rate of germination and to decrease the number of seeds which germinated. With dicotyledonous species, concentrations of 1.0 p.p.m. or greater caused swelling of both the hypocotyls and the roots, particularly in the region of the root-hypocotyl junction. Longitudinal ruptures of the surface frequently occurred in that region. The formation of lateral roots of dicotyledonous plants was inhibited completely by concentrations of 1.0 p.p.m. or more of 2,4-dichlorophenoxyacetic acid. The response of cereals was somewhat different. The most striking symptoms were curvatures of the coleoptile and amorphous gall-like growths on the roots, particularly at the root tip. The plumules did not emerge from the coleoptile when cereals were treated with 1.0 p.p.m. or more of 2,4-dichlorophenoxyacetic acid. Dissection of the coleoptiles showed that no development of the plumule had occurred.

No threshold concentration above which germination did not occur was apparent. Instead, the delay in the initiation of growth increased proportionally with increasing concentrations of 2,4-dichlorophenoxyacetic acid. Similarly, the number of abnormal seedlings and the severity of the symptoms increased gradually as the concentration was increased. As a result, no clear-cut distinction was possible between seeds which germinated and those which did not germinate, so that values for percentage of germination could not be assigned.

Quantitative measures of the inhibition of roots and shoots of spring wheat and cowpeas, given in table 1, were typical for the twenty-two cereal and broadleaf species treated with 2,4-di-

[1] Studies conducted at Camp Detrick, Frederick, Md., from February, 1944, to September, 1945, under the supervision of Dr. A. G. Norman.

chlorophenoxyacetic acid. These data confirm the observation that inhibition was in direct proportion to the concentration. They also show that the roots of wheat and cowpeas were more sensitive than the shoots. This was likewise true in all other species tested. The germination and seedling development of a few species, particularly sorghum, peas, and navy beans, deviated from the usual response in that concentrations of 0.01 and 0.1 p.p.m. appeared to be stimulating.

TABLE 1

THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID UPON YOUNG SEEDLINGS OF SPRING WHEAT AND COWPEAS GROWN IN MOIST CHAMBERS*

CONCENTRATION OF 2,4-DICHLOROPHENOXYACETIC ACID (P.P.M.)	WHEAT		COWPEAS	
	Length of root (mm.)	Length of shoot (mm.)	Length of root (mm.)	Length of shoot (mm.)
0	60.7	44.6	47.7	29.4
0.01	48.0	42.8	40.0	23.6
0.1	48.7	43.3	29.4	22.4
1.0	20.2	35.0	12.7	13.7
10.0	10.0	22.1	3.0	9.4
100.0	1.0	3.4	1.6	8.2

* Mean of four or more dishes, each containing approximately twenty-five seeds.

TABLE 2

EFFECT OF APPLICATIONS OF SOLUTIONS OF 10 P.P.M. OF SEVERAL GROWTH-REGULATORS UPON THE GROWTH OF ROOTS OF MAIZE GROWN IN MOIST CHAMBERS*

Compound	Root length (mm.)
4-chlorophenoxyacetic acid	42
2,4-dichlorophenoxyacetic acid	19
2-methyl-4-chlorophenoxyacetic acid	19
2,4,5-trichlorophenoxyacetic acid	20
Isopropyl-N-phenylcarbamate	78
Control	129

* Mean of four dishes, each containing approximately twenty-five seeds.

A comparison of the effect of 2,4-dichlorophenoxyacetic acid with three other chlorophenoxyacetic acids and isopropyl-N-phenylcarbamate upon the germination of maize is given in table 2. The symptoms produced by 4-chlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid were the same as those caused by 2,4-dichlorophenoxyacetic acid. However, 4-chlorophenoxyacetic acid was less effective than the other compounds. Isopropyl-N-phenyl carbamate did not cause curvatures or gall-like growths, nor did it inhibit growth as much as the chlorophenoxyacetic acids.

STUDIES IN SOIL

The results in moist chambers were compared with soil applications by preparing solutions which contained 0.78, 1.56, 3.13, 6.25, 12.5, 25, and 50 mg. of 2,4-dichlorophenoxyacetic acid per 50 ml. of 0.5 per cent Carbowax solution and applying these solutions to pots which contained about 1 pound of soil. After 3 days, the soil was removed, thoroughly mixed, replaced in the pots, and seeds of kidney beans, wheat, or oats were planted. Nine days after planting, when kidney-bean plants in the untreated pots were about 5 inches tall and wheat and oat plants 1–2 inches tall, all seedlings were removed from the soil and examined. At that time no emergence had occurred in pots treated with 6.25 mg. or more of 2,4-dichlorophenoxyacetic acid.

KIDNEY BEANS.—The effect of 2,4-dichlorophenoxyacetic acid upon kidney beans is shown in figure 1. When 3.12 mg. or more of the growth-regulator were applied, normal roots were severely inhibited; but 1.56 and 0.78 mg. appeared to stimulate growth of roots. However,

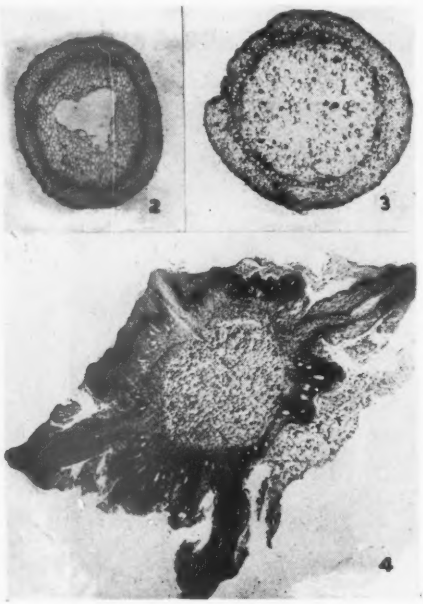
the shoots were stunted by even the lowest rate of application.

Histological studies of some of these plants, with methods described previously (2), showed that 2,4-dichlorophenoxyacetic acid had caused abnormalities to develop in the anatomy of the seedlings. In the control plants the hypocotyl was hollow as a result of the splitting of the pith. The strands of primary xylem and phloem were clearly developed and discontinuous; and differentiation of the cambium had occurred, although no secondary tissues were as yet laid down (fig. 2). With applications of 2,4-dichlorophenoxyacetic acid at rates of 1.56 and 3.13 mg. per pound of soil, a noticeable shortening of the hypocotyl was evident. Histologically, four differences were noted in transverse sections (fig. 3). These were (1) slight delay in the maturation of the primary conductive tissues, (2) failure of the pith to split to give rise to a hollow stem, (3) enlargement of the cortical and pith cells, and (4) slight stimulation of cell proliferation in regions of the primary phloem and the rays. The cambium was not clearly defined, but an area of actively dividing cells between the primary xylem and the phloem in the vascular bundles might have been interpreted as cambial in nature. Inability to distinguish between the inner cortical cells and the endodermis made observations in this region difficult and uncertain, but scattered proliferation had occurred. Where recognizable, the pericycle seemed unaffected by the 2,4-dichlorophenoxyacetic acid.

With higher rates of application of 2,4-dichlorophenoxyacetic acid, especially when more than 12.5 mg. per pound of soil were applied, there was increased stunting and distortion of the hypocotyl

FIG. 1.—Kidney beans germinated in soil treated with 2,4-dichlorophenoxyacetic acid. *C*, control; *1*, 25 mg.; *2*, 12.5 mg.; *3*, 6.25 mg.; *4*, 3.12 mg.; *5*, 1.56 mg.; and *6*, 0.78 mg. per pot.

region, with marked hypertrophy of the adventitious root system. The pith was solid, and there was considerable inhibition of the differentiation of conductive tissue and pericyclic fibers. Most striking of all was the development of fasciated roots, which generally possessed a te-



FIGS. 2–4.—Transverse sections of the hypocotyl of kidney bean. Fig. 2, untreated. Fig. 3, treated with 1.56 mg. of 2,4-dichlorophenoxyacetic acid per pound of soil. Solid pith and increased diameter caused by cell proliferation and enlargement. Fig. 4, treated with 50 mg. of 2,4-dichlorophenoxyacetic acid per pound of soil. Solid pith, root primordia, and disrupted cortex.

trarch arrangement. The development of the primary root was nearly completely inhibited. Figure 4 shows a transverse section through the hypocotyl after treatment with 2,4-dichlorophenoxyacetic acid at 50 mg. per pound of soil. The larger adventitious roots arose from, and over, the region of the medullary rays; but the primary phloem, and possibly the cambium, appeared to contribute cellular elements as well as the rays. Between the larger roots there extended broad proliferated areas, which generally consisted of cells derived from both ray and phloem elements and which sometimes gave rise to other smaller root primordia. These, as well as the apices of the larger roots, consisted of many small, deeply staining cells, which appeared to have undergone very rapid cell proliferation. No vascular differentiation occurred in the roots other than the formation of a few scattered and disorganized tracheid-like cells and some elongation of the cells making up the central core. The cortex was disrupted by the outward development of root primordia (fig. 4).

WHEAT AND OATS.—The effect of the 2,4-dichlorophenoxyacetic acid upon wheat and oats was to cause curvature and stunting of the coleoptiles (figs. 5 and 6) and severe inhibition of the roots. The development of the roots was more affected than that of the coleoptiles. Although an application of 0.78 mg. had little or no effect upon the coleoptiles, it caused severe abnormalities in root development (figs. 5 and 6). Applications of 6.25 mg. or more prevented all development of the roots.

Further tests of the effect upon germination of 2,4-dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyaceticacid,and isopropyl-N-phenylcarbamate were made upon soybeans, turnips, buckwheat, and barley, grown in standard greenhouse flats. The compounds were applied to the soil in 100 ml. of aqueous solution in amounts to give the equivalent of applications of 2 or 5 pounds per acre. Three days later the soil was removed, thoroughly mixed, replaced in the flats, and the seeds planted.

Six days after planting, normal emergence had occurred in all control flats. No

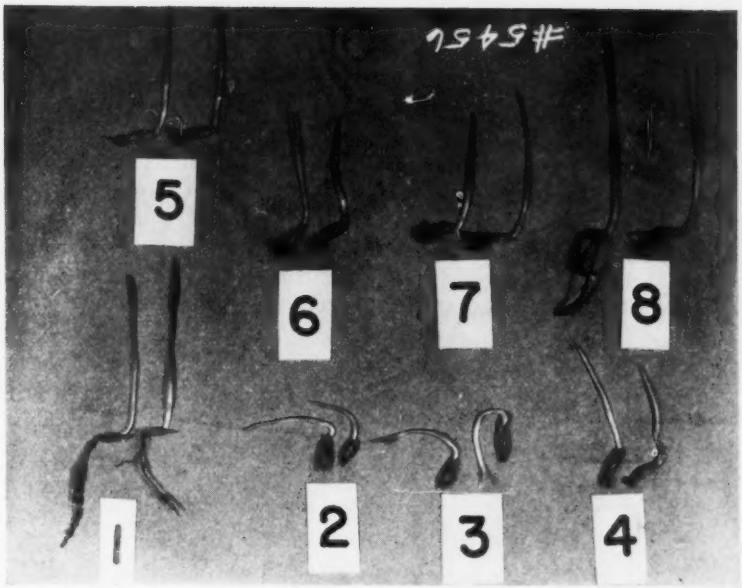


FIG. 5.—Oat seedlings germinated in soil treated with 2,4-dichlorophenoxyacetic acid. *1*, control; *2*, 50 mg.; *3*, 25 mg.; *4*, 12.5 mg.; *5*, 6.25 mg.; *6*, 3.13 mg.; *7*, 1.56 mg.; and *8*, 0.78 mg. per pound of soil.

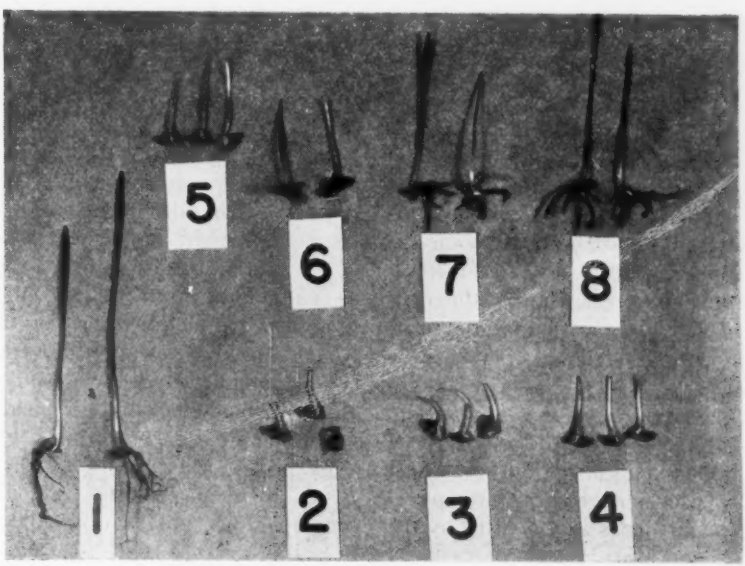


FIG. 6.—Wheat seedlings germinated in soil treated with 2,4-dichlorophenoxyacetic acid. *1*, control. *2*, 50 mg.; *3*, 25 mg.; *4*, 12.5 mg.; *5*, 6.25 mg.; *6*, 3.13 mg.; *7*, 1.56 mg.; and *8*, 0.78 mg. per pound of soil.

emergence of turnips or soybeans had occurred where 2,4-dichlorophenoxyacetic acid or 2-methyl-4-chlorophenoxyacetic acid had been applied; and, although some buckwheat and barley plants had emerged, the plants were stunted, particularly at the 5-pound rate of application. Isopropyl-N-phenylcarbamate had no noticeable affect upon the turnips or soybeans but had prevented emergence of any buckwheat or barley plants.

TABLE 3

GERMINATION OF THREE DICOTYLEDONOUS AND ONE CEREAL SPECIES TREATED WITH TWO CHLOROPHENOXYACETIC ACIDS AND ISOPROPYL-N-PHENYCARBAMATE, APPLIED TO THE SOIL IN AQUEOUS SOLUTIONS

COMPOUND	EQUIVALENT RATE PER ACRE (POUNDS)	NUMBER OF PLANTS*			
		Soybean	Buckwheat	Turnip	Barley
Control		11	22	41	14
2,4-dichlorophenoxyacetic acid	2	5	19	0	13
2-methyl-4-chlorophenoxyacetic acid	2	1	19	0	14
Isopropyl-N-phenylcarbamate	2	12	0	38	0
Control		13	20	39	15
2,4-dichlorophenoxyacetic acid	5	0	14	0	8
2-methyl-4-chlorophenoxyacetic acid	5	0	13	0	12
Isopropyl-N-phenylcarbamate	5	16	0	38	0

* Mean of four standard greenhouse flats.

Sixteen days after germination, a few badly stunted turnip and soybean plants had emerged from flats treated with 2,4-dichlorophenoxyacetic acid or 2-methyl-4-chlorophenoxyacetic acid, but the emergence of buckwheat and barley was nearly complete, though the plants were stunted.

The emergence 24 days after planting, given in table 3, shows the selectivity of the compounds. Although the buckwheat and the barley emerged nearly completely, the entire plants were stunted by the chlorophenoxyacetic acids, particularly the roots, which were short, swollen, and frequently covered by gall-like outgrowths. Buckwheat, a dicotyledonous plant, thus behaves like cereals in its response to chlorophenoxyacetic acids and isopropyl-N-phenylcarbamate.

When the selective herbicidal properties of 2,4-dichlorophenoxyacetic acid and other phenoxyacetic acids for broadleaf species and isopropyl-N-phenylcarbamate for grass species became apparent, trials were initiated to test for any general herbicidal properties of mixtures of the two types of compounds. Because it was also considered possible that the compounds might complement the action of one another, the experiments were designed so that interactionary effects, if present, could be determined statistically. The treatments applied were: (*A*) 2,4-dichlorophenoxyacetic acid, (*B*) 2,4,5,-trichlorophenoxyacetic acid, and (*C*) isopropyl-N-phenylcarbamate, all at 3 mg. per pot; mixtures of $A \times B$, $A \times C$, $B \times C$, all at a rate of $1\frac{1}{2}$ mg. of each compound per pot; and a mixture of $A \times B \times C$ at 1 mg. of each compound per pot. All treatments were applied to the soil in 25 ml. of water.

Oats were used as a representative of the grasses, and soybeans for the broadleaf species. Three series of tests were conducted. In the first series, twenty-five oat or 6 soybean seeds were planted per pot, and four pots of each species were given each treatment immediately after seeding. In the second and third series growth from each pot was used as a replicate in the analysis of the data.

It is readily seen from table 4 that 2, 4-dichlorophenoxyacetic acid and 2,4,5,-trichlorophenoxyacetic acid greatly decreased the growth of soybeans but did not cause significant reduction in the growth of oats; the converse was true for

TABLE 4

FRESH VEGETATIVE WEIGHT (IN GM.) OF OATS AND SOYBEANS TREATED WITH THREE GROWTH-REGULATORS AND MIXTURES OF THE GROWTH-REGULATORS APPLIED IN AQUEOUS SOLUTION TO THE SOIL

Compound	Rate in mg. per pot	Stage at time of treatment					
		Oats*		Soybeans*		Oats†	Soybeans†
		Seeding	Established	Seeding	Established	Established	Established
Control	0	11.55	8.56	12.72	12.32	1.60	1.87
2,4-dichlorophenoxyacetic acid	3	9.41	10.02	8.34‡	7.70‡	1.53	0.83‡
2,4,5-trichlorophenoxyacetic acid	3	9.99	9.60	6.66‡	5.28‡	1.32§	0.80‡
Isopropyl-N-phenylcarbamate	3	4.23‡	2.52‡	13.59	10.93	0.28‡	1.65
2,4-dichlorophenoxyacetic acid *plus* 2,4,5-trichlorophenoxyacetic acid	1½+1½	10.68	7.68	10.46	6.63‡	0.21‡	0.41‡
2,4-dichlorophenoxyacetic acid *plus* Isopropyl-N-phenylcarbamate	1½+1½	8.10‡	2.18‡	10.22	12.64	0.28‡	0.96‡
2,4,5-trichlorophenoxyacetic acid *plus* isopropyl-N-phenylcarbamate	1½+1½	4.67‡	1.99‡	9.87	8.55‡	0.29‡	0.98‡
2,4-dichlorophenoxyacetic acid *plus* 2,4,5-trichlorophenoxyacetic acid *plus* isopropyl-N-phenylcarbamate	1+1+1	7.99‡	3.44‡	10.49	7.25‡	0.23‡	0.23‡

* Mean of four pots.

† Mean of eight pots.

‡ Lower than the control at odds exceeding 1:19.

§ Lower than the control at odds exceeding 1:99.

an excess of seeds was planted, and the pots were thinned to fifteen oat plants or six soybean plants immediately before treatment, at which time the oats were 3 inches tall and the soybeans 2 inches tall. Four pots were given each treatment in series 2, and eight pots were given each treatment in series 3. The pots were subirrigated to avoid leaching. Twenty-three days after treatment the aerial portions of the plants were cut and weighed. The weight of fresh aerial

isopropyl-N-phenylcarbamate. The effects of the mixtures were not uniform in the three series of experiments; but, in general, mixtures of either of these chlorophenoxyacetic acids with isoprophyl-N-phenylcarbamate were effective in decreasing the growth of both oats and soybeans (table 4).

Analysis of the interactionary effects showed that the greater portion, if not all, of the effect upon oats was caused by isopropyl-N-phenylcarbamate, that the

phenoxyacetic acids similarly caused the greater portion of the decrease in growth of the soybeans, and that any interactions of the compounds were unimportant. These results indicated that isopropyl-N-phenylcarbamate and 2,4-dichlorophenoxyacetic or 2,4,5-trichlorophenoxyacetic acids are not complementary in action.

Discussion

In general, 2,4-diclorophenoxyacetic acid inhibited germination, decreased the growth of young seedlings, and caused abnormalities in the anatomy of the seedlings of twenty-two crop plants. More limited tests indicated that 4-chlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, and 2-methyl-4-chlorophenoxyacetic acid produced the same responses in germinating seeds.

In moist chambers the inhibition of growth of germinating cereal and dicotyledonous seeds was not greatly different over a wide range of concentrations of 2,4-dichlorophenoxyacetic acid. However, chlorophenoxyacetic acids applied to the soil nearly completely prevented the germination and establishment of broadleaf species at rates of application which merely stunted cereals. The behavior of isopropyl-N-phenylcarbamate was quite different, in that it had much less effect upon germinating cereal seeds in moist chambers than chlorophenoxyacetic acids, but it completely prevented the establishment of cereals in soil.

The foregoing results show that the specificity of certain growth-regulating compounds may be different when applied to germinating seeds and to established plants, and they may also indicate some influence of the soil upon the response obtained. Consequently, caution is necessary in evaluating the effectiveness of a weed-killer upon any single species on the basis of germinating-seed tests in moist chambers or upon tests made at a single stage of development.

In the soil a mixture of either 2,4-dichlorophenoxyacetic acid or 2,4,5-trichlorophenoxyacetic acid with isopropyl-N-phenylcarbamate proved an effective herbicide against young seedlings of both broadleaf and cereal species. However, mixtures of these compounds were not more effective on soybeans and oats than the compounds alone, and evidence of clear-cut interactionary or complementary effects was not obtained.

The lack of specificity of 2,4-dichlorophenoxyacetic acid in inhibiting the germination of seeds of twenty-two crop plants, which indicated a more general herbicidal effect upon very young than upon older plants, may have practical application in weed-control programs.

Summary

1. In studies in moist chambers, 2,4-dichlorophenoxyacetic acid was found to delay germination and to cause abnormalities in the seedling development of twenty-two broadleaf and cereal species. Isopropyl-N-phenylcarbamate was less effective than four chlorophenoxyacetic acids in reducing growth of seedlings of maize.

2. 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid applied to the soil prevented the germination of broadleaf species at rates which stunted cereals but did not prevent establishment. Isopropyl-N-phenylcarbamate prevented the establishment of cereals at rates which had no effect upon broadleaf species. Buckwheat, a dicotyledonous species, responded like a cereal.

3. Mixtures of chlorophenoxyacetic acids with isopropyl-N-phenylcarba-

mate inhibited the development of seedlings of both broadleaf and cereal species and hence have promise as general herbicides, but they gave no evidence of complementary action of the two types of compounds.

4. The general lack of specificity of 2,4-dichlorophenoxyacetic acid in inhibiting the germination of seeds of twenty-two different species may have practical applications in weed-control programs.

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PERSISTENCE OF SOME PLANT GROWTH-REGULATORS WHEN APPLIED TO THE SOIL IN HERBICIDAL TREATMENTS[1]

H. ROBERT DEROSE, CAPT., A.U.S.

Introduction

The herbicidal action of the halogenated phenoxyacetic acids has been amply demonstrated. The possibility that some compounds of this series also possess a selectivity of action on certain weed species further emphasizes the importance of the phenoxyacetic acids in any weed-control program (3). Their judicious use, however, depends upon a knowledge of their action under many and varied conditions.

Since with such compounds it is possible to achieve herbicidal effects by treatment of the soil, as well as by application of aqueous or oil solutions to the plant itself, it is of importance to determine their behavior following incorporation into the soil. SLADE, TEMPLEMAN, and SEXTON (2) demonstrated that 2-methyl-4-chlorophenoxyacetic acid, an excellent herbicidal compound, persisted for some time in the soil. NUTMAN, THORNTON, and QUASTEL (1), on the other hand, found that 2,4-dichlorophenoxyacetic acid, when applied to the soil in small amounts, lost its toxicity completely within 3–6 days. They also demonstrated that leaching equivalent to 1.4 inches of water resulted in a loss of activity that was just significant, but that far greater volumes of water did not completely remove this compound from the soil. Reduction in herbicidal effectiveness or total loss in activity of the herbicide can result from leaching, from hydrolysis or decomposition due to the action of the micro-organisms of the soil, or from inactivation due to adsorption or fixation by soil colloids.

The investigations reported below are

[1] Studies conducted at Camp Detrick, Frederick, Md., from October, 1944, to September, 1945, under the supervision of Dr. A. G. Norman.

the results of studies undertaken to determine the persistence of several growth-regulators when added to the soil.

Experimentation and results

GREENHOUSE EXPERIMENTS

EXPERIMENT 1.—The purpose of this experiment was to determine whether 2,4-dichlorophenoxyacetic acid is readily leached from a greenhouse soil. A stock solution was prepared by dissolving 1 gm. of the chemical in 5 gm. of melted Carbowax 1500 and adding this immediately, drop by drop, to 1 liter of warm water. Solutions containing various amounts of 2,4-dichlorophenoxyacetic acid were prepared by dilution from the stock solution. Half-gallon glazed crocks, which had a glass-wool filter placed at the drainage outlet, were used for soil treatment. The drainage outlet was fitted with a one-hole rubber stopper, which had a small glass tube inserted in order to allow collection of the leachate in Erlenmeyer flasks. The crocks were filled with 2500 gm. of soil, consisting of a mixture of one part silt loam and one part medium sand. Solutions of 2,4-dichlorophenoxyacetic acid containing 100, 50, 12.6, 6.4, or 3.2 mg., respectively, were added to the crocks. After the soil had been treated with the growth substance or herbicide, enough additional water was applied to bring the soil to its predetermined water-holding capacity. It was assumed that in this way the 2,4-dichlorophenoxyacetic acid would be equally distributed throughout the soil mass. After treatment, the soil was allowed to stand 2 days before leaching.

Each series of crocks was then leached with an amount of water equivalent to 1.5, 3.0, or 4.5 inches of rain. The leachate was collected and tested for toxicity by placing one drop on young red kidney bean and tomato leaves at the junction of the petiole and the expanded leaf blade. The leached soils, after partial drying, were tested for residual 2,4-dichlorophenoxyacetic acid by observing the growth responses of young tomato plants transplanted into them.

Parts of the leachates from the soils which had been leached with water equivalent to 1.5, 3.0, or 4.5 inches of rain were combined and, after filtration, were allowed to evaporate slowly so that the growth substance could crystallize. It was found that white needle-like crystals were formed. These crystals were dissolved in boiling benzene, an equal amount of petroleum ether added, the solution cooled to 0° C., and melting-point determinations were made on the recrystallized product. It was found in each case that the recrystallized compound had a melting-point of 139° C. (uncorrected), which is approximately the melting-point of 2,4-dichlorophenoxyacetic acid.

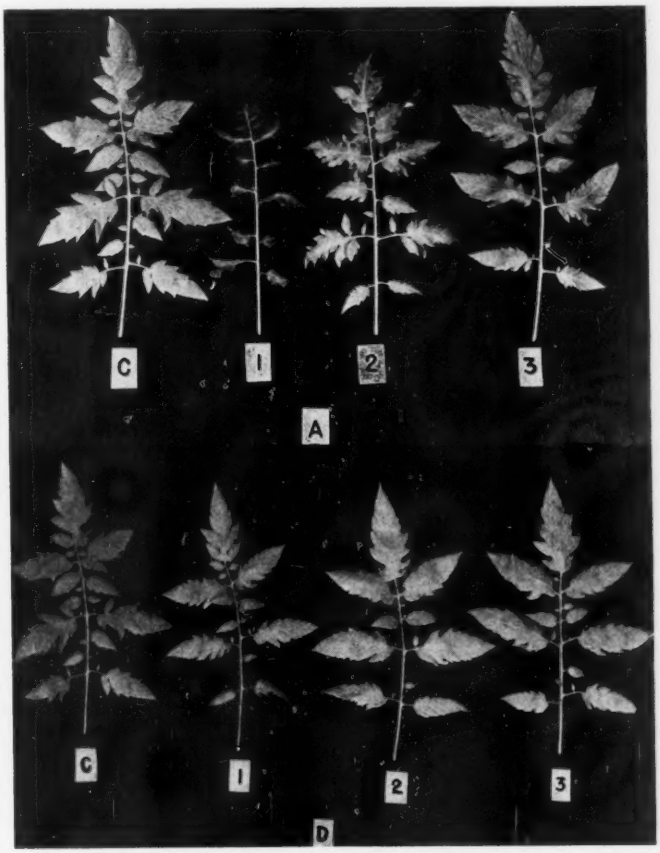
When one drop of an 0.5% Carbowax aqueous solution containing a small amount of the recrystallized compound was placed at the junction of the petiole and the expanded leaf blades of young tomato and bean plants, stem curvature and epinasty characteristic of plants treated with 2,4-dichlorophenoxyacetic acid were obtained within a few hours. Similar reactions occurred when the leachates themselves were tested for toxicity.

It was further found that when young tomato plants were transplanted into soils which had been treated initially with 100 mg. of the herbicide per pot and leached with 1.5 and 3.0 inches of water, respectively, plant responses characteristically caused by 2,4-dichlorophenoxyacetic acid developed (fig. 1). On the other hand, if the soil was leached

with 4.5 inches of water, there were no plant responses due to the residual growth substance. It was further found that when young tomato plants were transplanted into soils which had been treated with 3.2 mg. of 2,4-dichlorophenoxyacetic acid and leached with 1.5, 3.0, and 4.5 inches of water, respectively, normal leaves were produced (fig. 2).

It may therefore be concluded that the 2,4-dichlorophenoxyacetic acid was leached from the soil or that, if any of the growth-regulator remained in the soil, it was inactivated in some manner as yet undetermined.

EXPERIMENT 2.—Because of the nature of the information obtained in Experiment 1, it seemed pertinent to study



FIGS, 1, 2.—Fig. 1, Effects of residual 2,4-dichlorophenoxyacetic acid upon the leaves of young tomato plants. The soil had been treated with 100 mg. of compound per pot and leached with water equivalent to various amounts of rainfall prior to transplanting. *C*, control, 1–1.5, 2–3.0, and 3–4.5 inches of rainfall. Fig. 2, effects of residual 2,4-dichlorophenoxyacetic acid upon the leaves of young tomato plants. The soil had been treated with 3.2 mg. of chemical per pot and leached with water equivalent to various amounts of rainfall prior to transplanting. *C*, control, 1–1.5, 2–3.0, and 3–4.5 inches of rainfall.

the persistence of 2,4-dichlorophenoxyacetic acid in soil which was not subjected to leaching. A greenhouse experiment which ran for 12 weeks was set up for this investigation. A stock solution was prepared by dissolving 390 mg. of purified 2,4-dichlorophenoxyacetic acid in 650 ml. of 0.5% Carbowax aqueous solution. Several series of half-gallon pots were filled with greenhouse soil (5 pounds to each pot), and the stock solution of 2,4-dichlorophenoxyacetic acid added in amounts necessary to give 1, 2, 3, 4, 5, 6, 8, 10, or 12 mg. per pound of soil. After the soil had been treated, 150 ml. of water were added to each crock in order to distribute the solution through the soil mass. The soil was not subjected to leaching but was kept just moist during the period that was allowed to elapse before planting.

Prior to planting, the soil was partially dried, removed, thoroughly mixed, and replaced in the pots to avoid the possibility of an unequal distribution of 2,4-dichlorophenoxyacetic acid. At intervals of 4 weeks, red kidney-bean seeds were planted and one tomato plant, 10 days old, was transplanted into each pot. A new series of pots was started every 2 weeks, so that, by comparison of plant responses, information could be obtained as to the rate of decreasing effectiveness of the herbicide.

Initially, it was found that tomato plants were unable to establish themselves in any of the treated pots. After the 8th week, however, even at the higher application rates of 4–12 mg. per pound of soil, normal plants developed. At 6 weeks, plants developed normally only if the original application was 3 mg. per pound of soil or less, and at 4 weeks only if the initial application did not exceed 1 mg. per pound of soil. The observations with respect to seed germination were similar. After a period of 6 weeks, all seeds germinated, no matter how high the initial application had been. At 4 weeks, only about 50% germination was obtained if the initial application of 2,4-dichlorophenoxyacetic acid was 4 mg. per pound of soil or higher. It therefore appears that relatively heavy applications of 2,4-dichlorophenoxyacetic acid are likely to become inactivated in soil in 6–8 weeks. These experiments, it is to be emphasized, were carried out under greenhouse conditions, with watering adjusted so that no drainage losses from the pots occurred.

Field experiments

These experiments were carried out on a silt-loam soil with a pH of 6.5. The soil had been cultivated the previous year, and the field was prepared for crops according to the usual agronomic practices.

Experiment 1.—The object of this experiment was to compare the persistence of several growth substances when applied to a field soil. Plots (10 × 20 feet) were prepared and made approximately level. Around each plot a small dike was constructed to prevent surface washing. Lengthwise through the center of each plot a small dike was similarly constructed to divide it into a treated area and an untreated control area. In the design of the experiment an untreated control plot was placed adjacent to a treated plot.

Just prior to planting, the seed beds were carefully prepared and the growth substances applied at the excessively heavy rate of 50 pounds per acre. The growth substances tested were 2,4-dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, and isopropyl N-phenylcarbamate. Measured amounts of these substances were added to 200-gm. portions of Fuller's earth, and these mixtures were dusted on the soil. After

the applications had been made, the surface layer of soil was carefully raked.

Soybeans were used as the test crop. Sixty seeds were planted in rows 5 feet long and 14 inches apart. New plantings were made during the experiment at 17-day intervals after the initial planting.

The results of this investigation are summarized in figure 3. It is to be noted that 34 days after the initial application there was enough residual growth substance remaining in the soil to cause total inhibition of germinating soybeans. At 51 days, about 40% of the soybeans survived in the soil which had been treated with isopropyl N-phenylcarbamate; and there was no germination in the soil which had been treated with either 2,4-dichlorophenoxyacetic acid or 2-methyl-4-chlorophenoxyacetic acid. On the other hand, 90 days after the soil had been treated with 2,4-dichlorophenoxyacetic acid or isopropylphenylcarbamate there was more than 90% survival of soybean plants. There was, then, less than 25% survival of soybean plants in the soil which had been treated with 2-methyl-4-chlorophenoxyacetic acid. During the period of the investigation, several heavy rains occurred, which may have leached considerable amounts of the growth-regulators from the soil.

EXPERIMENT 2.—In this study a comparison of persistence was made between two growth substances on two different test crops. The soil was prepared as in experiment 1 and the same rate of application was used. The growth substances tested were 2,4-dichlorophenoxyacetic acid and isopropylphenylcarbamate, and the crops were soybeans and oats.

It was found that, as measured by the germination and survival of oats and despite the excessively high rate of application of 50 pounds per acre, isopropylphenylcarbamate did not persist in the soil for more than 60 days (table 1). On the other hand, as measured by residual toxicity toward soybeans, 2,4-dichlorophenoxyacetic acid was more persistent in soil, though, after 80 days, the germination of soybeans was almost normal.

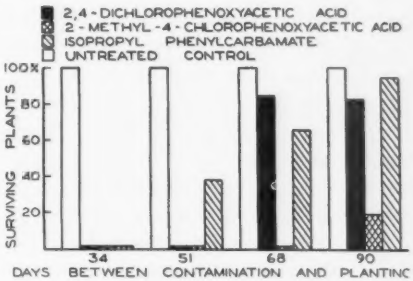


FIG. 3.—Persistence in the soil of three growth-regulating substances. The compounds were applied at the rate of 50 pounds per acre, and germination and survival of soybeans were used to indicate presence of compound in the soil.

TABLE 1

COMPARISON OF THE PERSISTENCE OF TWO GROWTH SUBSTANCES WHEN ADDED TO THE SOIL UNDER FIELD CONDITIONS AS MEASURED BY THE GERMINATION OF SOYBEANS (2,4-DICHLOROPHENOXYACETIC ACID) AND OATS (ISOPROPYL N-PHENYLCARBAMATE)

TREATMENT	PERCENTAGE OF GERMINATION AND SURVIVAL AFTER				
	10 days	26 days	40 days	60 days	80 days
2,4-dichlorophenoxyacetic acid	0	0	0	75	89
Control	96	94	98	100	100
Isopropylphenylcarbamate	0	20	45	95	98
Control	93	98	100	96	97

EXPERIMENT 3.—The purpose of this experiment was to observe the persistence of 2,4-dichlorophenoxyacetic acid

and 2,4,5-trichlorophenoxyacetic acid in soil which had produced a crop of potatoes.

The soil of a plot of Irish potatoes had been treated with these growth substances at the rates of 3, 10, and 20 pounds per acre. About 90 days after the potatoes had been planted and 10 days after harvesting, the field was carefully disked and replanted to soybeans. It was found that there were no residual effects from the 3-pound per acre application of either 2,4-dichlorophenoxyacetic acid or 2,4,5-trichlorophenoxyacetic acid. However, in the cases of 10- and 20-pound applications, 2,4,5-trichlorophenoxyacetic acid showed greater residual effects on soybeans than did 2,4-dichlorophenoxyacetic acid. Total inhibition of soybean germination was produced by 2,4,5-trichlorophenoxyacetic acid when applied originally at 20 pounds per acre, while 2,4-dichlorophenoxyacetic acid at the same rate did not produce a noticeable effect.

Discussion

The experiments described above revealed (1) that 2,4-dichlorophenoxyacetic acid and isopropylphenylcarbamate, even when applied in excessive amounts, readily leached from the soil and that when leaching was prevented, the former compound was inactivated; and (2) that 2-methyl-4-chlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid are much more persistent, and were herbicidally active for a longer period of time than was 2,4-dichlorophenoxyacetic acid. The data tend to suggest, therefore, that, when using a herbicide as a soil contaminant, proper selection of the herbicide is important. The 2,4-dichlorophenoxyacetic acid is effective for a short time but rapidly loses its effectiveness through leaching or inactivation. The 2-methyl-4-chlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid, on the other hand, are equally effective in inhibiting growth and germination but differ in being effective over a greater period of time. At the present time no information is available to explain this difference in persistence.

Summary

1. Greenhouse experiments for determining the leachability and persistence of 2,4-dichlorophenoxyacetic acid in soil have been described.

2. When a soil was contaminated with 2,4-dichlorophenoxyacetic acid and then leached, it was found that the compound was present in the leachate.

3. It was demonstrated that normal plants developed in a greenhouse soil 8 weeks after the latter had been treated with high rates of 2,4-dichlorophenoxyacetic acid. Observations with respect to seed germination were similar. It may therefore be concluded that, under greenhouse conditions, this herbicide does not remain active in unleached soil for longer than 8 weeks.

4. In the field it was shown that 2,4-dichlorophenoxyacetic acid did not persist in soil for more than 80 days after treatment, while isopropyl N-phenylcarbamate had apparently disappeared within 60 days after treatment.

5. A field comparison was made of the persistence of 2,4-dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, and isopropylphenylcarbamate applied at a heavy rate to the soil. It was found that after 68 days the soil still contained enough 2-methyl-4-chlorophenoxyacetic acid to be toxic to soybeans. On the other hand, 68 days after the soil had been treated with 2,4-dichlorophenoxyacetic acid or isopropylphenylcarbamate, there was practically a complete

disappearance of the herbicides from the soil even when the compounds were applied at rates far above those likely to be used in practice.

6. 2,4,5-trichlorophenoxyacetic acid retains its herbicidal effectiveness in the soil for a longer period of time than does 2,4-dichlorophenoxyacetic acid.

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THE ACTION OF ISOPROPYLPHENYL-CARBAMATE UPON PLANTS[1]

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The physiological activity of compounds of the urethane series upon animals has been known for over thirty years (**1**). The first known reference to experimentation to determine the effect of these compounds upon plants is that of FRIESEN (**2**), who reported the retardation of seed germination and abnormal growth of oat and wheat seedlings following treatment with dilute solutions of phenylurethane. Since 1929, LEFÈVRE (**4**), SIMONET and GUINOCHET (**5**), GUINOCHET (**3**), SWINGLE and MAYER (**6**), and THOMPSON *et al.* (**8**) have reported the effects of various urethanes upon plants. TEMPLEMAN and SEXTON (**7**) were the first to apply isopropylphenylcarbamate, which they found to be more active upon cereals than some other urethanes but without effect upon certain dicotyledonous species.

This paper presents the results from several greenhouse and field experiments conducted to compare the herbicidal properties of O-isopropyl N-phenylcarbamate with those of some of the more active phenoxyacetic acid derivatives.

[1] Studies conducted at Camp Detrick, Frederick, Md., from September, 1944, to September 1945, under the supervision of Dr. A. G. Norman.

Soil applications

EXPERIMENT 1

Aqueous solutions of isopropylphenylcarbamate, 2,4-dichlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid were applied to oats and soybeans. Twenty-five oat seeds or six soybean seeds were planted per 4-inch pot, which contained approximately 1 pound of soil. One group of pots was treated immediately after seeding with 3 or 6 mg. of the growth regulator applied to the soil in 25 ml. of water. The other group of pots was not treated until the oat plants were about 4 inches, and the soybeans 3 inches, in height, with the first trifoliate leaf partially unfolded. The second group was thinned to fifteen oat plants and three soybean plants before treatment. Care was taken to apply the solution

only to the soil and not upon the seedling plants. The pots were placed in saucers and subirrigated to prevent leaching of the chemicals. In both groups, four pots were given each treatment, and the green weight of the aerial portions of the plants in a single pot was used as a replicate in the analysis of the data. The plants were cut and weighed 23 days after treatment.

In untreated pots, twenty-one to twenty-five oat seeds germinated and made good growth. In pots treated with 6 mg. of isopropylphenylcarbamate, the coleoptiles were short and thickened at emergence. They became progressively darker blue-green in color during the first week following emergence. The tips of the coleoptiles then became brown. The necrosis progressed from the tip toward the base, and 23 days after treatment most had died without emergence of the plumule. In each pot one or two oat plants made some growth. This was apparently due to uneven distribution of the growth-regulator throughout the soil. The average weight of the aerial vegetative growth of these plants was about 14 per cent of that of the plants in the untreated pots (table 1).

The same effect, but to a lesser degree, was observed when oats were treated at seeding with 3 mg. of isopropylphenylcarbamate. Usually three to five plants made some growth. The fresh weight of the plants at time of harvest was about one-third that of the untreated controls (table 1).

When isopropylphenylcarbamate was applied to established oat plants, no effect was apparent for 2 or 3 days following treatment. After the third day the color became noticeably darker green, and this color intensification continued for about 2 weeks, during which period little or no elongation or further production of leaves occurred. At approximately 2 weeks after treatment, the leaves started to turn brown at the tips. The leaves progressively dried out from the tips, and nearly all plants were dead 23 days after treatment. Little difference was noted in the results produced by the 3- and the 6-mg. treatments. The weight of the aerial portions of the plants for

TABLE 1

THE EFFECT OF ISOPROPYLPHENYLCARBAMATE, 2,4-DICHLOROPHENOXYACETIC ACID, AND 2,4,5-TRICHLOROPHENOXYACETIC ACID UPON THE VEGETATIVE WEIGHT (IN GRAMS) OF OATS AND SOYBEANS, GROWN IN 4-INCH POTS, AND CUT 23 DAYS AFTER TREATMENT

Treatment	Rate (mg. per pot)	Stage at time of treatment			
		Oats		Soybeans	
		Seeding	4 inches	Seeding	4 inches
Isopropylphenylcarbamate	6	1.52*	2.28*	14.55	11.26
Isopropylphenylcarbamate	3	4.23*	2.52*	13.59	10.93
2,4-dichlorophenoxyacetic acid	6	9.84	7.89	6.05*	6.22*
2,4-dichlorophenoxyacetic acid	3	9.41	10.02	8.34*	7.70*
2,4,5-trichlorophenoxyacetic acid	6	8.75†	7.69	2.92*	3.18*
2,4,5-trichlorophenoxyacetic acid	3	9.99	9.60	6.66*	5.28*
Untreated control		11.55	8.56	12.72	12.32

* Weight of vegetative growth reduced below the untreated control (odds 99:1).

† Weight of vegetative growth reduced below the untreated control (odds 19:1).

both rates was about one-third that of the untreated controls (table 1).

The two phenoxyacetic acid derivatives, whether applied at time of seeding or to young established plants, caused slight chlorosis and slight stunting of growth but failed to reduce the weight of vegetative growth of oats to a statistically significant degree (except 6 mg. of 2,4,5-trichlorophenoxyacetic acid). Isopropylphenylcarbamate produced no noticeable effect upon the soybeans and did not reduce the weight of vegetative growth (table 1). The high toxicity of the two phenoxyacetic acid derivatives upon soybeans is apparent from table 1.

Experiment 2

To determine whether the apparent lack of physiological effect of isopropylphenylcarbamate on soybeans was general for broadleaf species, this compound was applied at rates of 5–18 mg. to soil in which seeds of several species had been planted. Four-inch pots were used, each containing about 1 pound of soil, and the compound was applied in 25 ml. of water. Germination and development of the seedlings of soybeans, kidney beans, cowpeas, sunflowers, turnips, radishes, and sugar beets were not affected. Tomatoes and potatoes are broadleaf species which may have been affected. Soon after treatment of 10-day-old tomato plants, treated plants became noticeably taller than the controls, although no proliferation or epinasty was observed. Two months after treatment, these plants were about 50 inches in height, whereas the control plants were approximately 40 inches in average height. When 40 mg. of isopropylphenylcarbamate were applied to potatoes about 3 inches in height, the primary stem became stunted and rosette-like within two weeks after treatment. Fifty-one days after treatment the rosette appearance of the primary stem persisted, but stems produced by the axillary buds appeared to be normal. The germination of buckwheat, *Fagopyrum esculentum*, was completely prevented by either 2 or 5 mg. of isopropylphenylcarbamate per 4-inch pot.

Experiment 3

This experiment was conducted to test the relative susceptibility of several cereal crops to the herbicidal activity of isopropylphenylcarbamate. Six cereals—oats, wheat, barley, corn, rice, and millet—were seeded in 4-inch pots. Pots of wheat, oats, barley, and rice were thinned to ten plants before treatment, millet to five plants, and corn to four plants. One and one-half or 3 mg. of isopropylphenylcarbamate were applied to the soil in 25 ml. of water 2 weeks after seeding, at which time the wheat, oats, corn, barley, and millet were about 2 inches tall and the rice about 1 inch tall. The pots were subirrigated subsequently. The fresh weight in grams of the aerial growth was taken 23 days after treat-

TABLE 2

THE EFFECT OF ISOPROPYLPHENYLCARBAMATE UPON THE VEGETATIVE GROWTH OF SIX CEREAL SPECIES. GREEN WEIGHT (TAKEN 23 DAYS AFTER TREATMENT) OF TREATED POTS IS GIVEN IN PERCENTAGE OF THE UNTREATED CONTROLS, BASED UPON THE MEAN WEIGHT OF FOUR POTS PER TREATMENT. CONTROL EXPRESSED AS 100%

Crop	Treatment Rate	
	1.5 mg. per 4-inch pot	3.0 mg. per 4-inch pot
Oats (*Avena sativa*)	30.1	22.7
Wheat (*Triticum vulgare*)	23.2	28.7
Barley (*Hordeum vulgare*)	61.7	42.2
Corn (*Zea mays*)	69.4	43.6
Rice (*Oryza sativa*)	78.4	77.0
Millet (*Setaria italica*)	165.6	97.8

ment. Four pots were given each treatment.

No effect was apparent upon millet. Although the weight of the vegetative growth would indicate stimulation where 1.5 mg. were applied (table 2), it is likely that this was due to random error. All the other cereals were severely stunted by isopropylphenylcarbamate and the typical dark blue-green color appeared, which had been previously observed in oats following application of this compound. A few plants of all cereals except millet had died by the 23d day after treatment. Examination of the data in table 2 shows that oats and wheat were more inhibited in growth by isopropylphenylcarbamate than were barley, corn, or rice.

TABLE 3

THE EFFECT OF ISOPROPYLPHENYLCARBAMATE COMPARED TO SOME PHENOXYACETIC ACID DERIVATIVES UPON THE PRODUCTION OF RICE GRAIN. THE MEAN WEIGHT (TAKEN AT MATURITY) OF FOUR CROCKS OF FIVE PLANTS TREATED WITH 62.5 MG. IS GIVEN (IN GRAMS)*

TREATMENT	YIELD OF GRAIN IN GRAMS				
	Replications				Mean
	1	2	3	4	
Isopropylphenylcarbamate	12.4	9.2	4.8	8.4	8.7
Ammonium 2-methyl-4-chlorophenoxyacetate	52.5	55.4	48.7	28.9	46.4
Ammonium 2,4-dichlorophenoxyacetate	46.1	54.0	61.7	48.1	52.5
1:1 mixture of isopropylphenylcarbamate and ammonium 2-methyl-4-chlorophenoxyacetate	17.3	24.5	18.3	11.6	17.9
Untreated control	68.8	52.7	56.7	89.2	66.9

* Minimum significant difference between treatment means is 5.4 gm. at the 5% level of significance and 7.6 gm. at the 1% level.

EXPERIMENT 4

Isopropylphenylcarbamate applied to the irrigation water of 30-day-old rice plants in flooded half-gallon crocks at 2.5–10 mg. per crock produced the typical dark-green color of foliage which characterized its effect upon younger nonflooded rice (expt. 3) and other cereals. However, 23 days after treatment the flooded rice had tillered profusely and was luxuriantly vegetative, with the leaves slender and dark green in color. The weight of the vegetative growth did not differ significantly from that of the untreated controls when harvested 23 days after treatment.

Further to test the effect of the compound upon rice, it was applied in aqueous solution to the irrigation water of rice about $3\frac{1}{2}$ months old at 10 or 30 mg. per 1-gallon glazed crock. Tillering was stimulated even in these older plants, but the tillers were somewhat stunted, maturity was delayed, and the panicles produced were small. The amount of grain was greatly reduced; the untreated control yielded 32.7 gm. per four pots, the 10-mg. treatment 22.2 gm., and the 30-mg. treatment 1.3 gm. Similar results were obtained from an experiment designed to compare isopropylphenylcarbamate with some of the phenoxyacetic acid derivatives. It is apparent from table 3, despite the recognized unreliability of greenhouse-yield results, that the former compound caused a greater reduction in grain yield of rice than the

phenoxyacetic acid derivatives against which it was compared.

Experiment 5

Two field trials were run in 1945 to test the herbicidal activity of isopropylphenylcarbamate upon plants under field conditions. Since insufficient amounts of isopropylphenylcarbamate were available for application at rates which were deemed desirable, it was decided to prepare a 1:1 mixture of this compound with 2,4-dichlorophenoxyacetic acid and compare the results with 2,4-dichlorophenoxyacetic acid alone. To facilitate a uniform distribution of these herbicides upon the soil, they were impregnated in an equal weight of oven-dry sawdust. This was accomplished by dissolving the 2,4-dichlorophenoxyacetic acid in an isopropyl alcohol–acetone mixture (1:1) and the mixture of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid in isopropyl alcohol. The solutions were poured over the sawdust, which was then thoroughly stirred, and the solvent evaporated at 45° C.

The two preparations were applied to winter rye (sown the previous fall) on April 14, when the rye was about 1 foot tall. Rates calculated at 3, 10, and 20 pounds per acre of herbicide were applied to plots 10 feet square, which were arranged in a complete randomized block-field design with each treatment in quadruplicate.

Two weeks after treatment those plots which had received 10 or 20 pounds per acre of the mixture of 2,4-dichlorophenoxyacetic acid and isopropylphenylcarbamate were stunted, dark green in color, and delayed in heading. By May 19 the plots treated with 2,4-dichlorophenoxyacetic acid alone were in full flowering stage, while plots which had received the mixture were stunted severely and had not started to head. The plots were harvested at maturity on July 27, and grain yield was taken in grams (table 4). Plots which had been treated with the mixture of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid were greatly reduced in grain yield. The reduction in yield increased directly

TABLE 4

A COMPARISON OF THE EFFECT UPON GRAIN YIELD (TAKEN AT MATURITY IN GRAMS) OF ISOPROPYLPHENYLCARBAMATE AND A MIXTURE OF ISOPROPYLPHENYLCARBAMATE AND 2,4-DICHLOROPHENOXYACETIC ACID APPLIED IN SAWDUST TO WINTER RYE 12 INCHES TALL*

Treatment†	Rate (pounds per acre)	Yield of grain in grams				
		Replications				Mean
		1	2	3	4	
2,4-dichlorophenoxyacetic acid	3	1001	658	820	954	858
2,4-dichlorophenoxyacetic acid	10	806	735	721	459	680
2,4-dichlorophenoxyacetic acid	20	632	721	490	684	631
Isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid (1:1 mixture)	3	530	228	475	457	422
	10	104	74	165	79	106
	20	43	0	0	0	11

* Minimum significant difference between treatment means is 53 gm. at the 5% level and 74 gm. at the 1% level of significance.
† The untreated control plots were lost during the threshing operation.

with increases of amount of the herbicide applied, with the differences between treatment rates highly significant statistically. Increases in the amount of 2,4-dichlorophenoxyacetic acid applied likewise resulted in smaller yields, but even the 20-pound per acre application was less effective in reducing yield of grain than the 3-pound per acre application of the mixture. It is readily apparent that isopropylphenylcarbamate was the more active portion of the mixture of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid.

TABLE 5

THE EFFECT OF ISOPROPYLPHENYLCARBAMATE AND 2,4-DICHLOROPHENOXYACETIC ACID UPON THE YIELD OF GRAIN (TAKEN AT MATURITY IN GRAMS) OF OATS. FIGURES REPRESENT THE MEAN OF FOUR PLOTS, 6×8 FEET*

Rate of Application (pounds per acre)	2,4-Dichlorophenoxyacetic acid	Mixture of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid (1:1)
	First application (planting)	
3	188	197
10	106	50
20	70	8
	Second application (2 inches tall)	
3	195	83
10	92	8
20	55	7
	Third application (prebooting)	
3	317	275
10	221	167
20	184	96
	Fourth application (heading)	
3	320	295
10	315	267
20	386	312
	Untreated controls	
0	316	370
0	327	360

* Minimum significant difference between treatment means is 53.2 gm. at the 5 per cent level of significance and 70.7 gm. at the 1% level.

Similar results were obtained when the same treatments were applied to seedling oats. Applications were made at four different stages of development—seeding, 2 inches tall, prebooting, and heading—to plots 6×8 feet in size, arranged in a complete randomized block design replicated four times. The data tabulated in table 5 show that the mixture of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid was much more active in inhibiting grain yield of oats than 2,4-dichlorophenoxyacetic acid. Again the more active portion of the mixture was the isopropylphenylcarbamate. Applications at planting time proved slightly less effective than applications to oat seedlings about 2 inches in height (table 5). Both early applications were more effective than the one made to plants about a foot in height. Very little effect was obtained from applications after the oats had reached the heading stage of development.

Spray trials

Several experiments were conducted to determine the effectiveness of spraying as a method of applying isopropylphenylcarbamate. The low solubility of isopropylphenylcarbamate in water, about 250 p.p.m. at 25° C., proved too low to make aqueous sprays practicable. Isopropylphenylcarbamate is insoluble in oil. However, it may be dissolved in twice its weight of tributylphosphate,

and the resulting solution is oil miscible in all proportions. Oil sprays in the greenhouse were applied in a spray chamber with an area of 0.5 square yards and 4 feet deep, with a DeVilbiss atomizer operated at a pressure of 15 pounds per square inch. Sprays in the field were applied with a DeVilbiss CV type nozzle, operated at 15 pounds pressure per square inch from a pressure cylinder. Field plots were protected from the effect of wind during the spray operation by means of canvas-covered frames about 4 feet high.

rate of 2 or 10 pounds per acre in 10 ml. of tributylphosphate-oil per square yard to the rye when it reached the prebooting stage of development. The isopropylphenylcarbamate at 10 pounds per acre caused rapid death of all plants in plots to which it was applied, and no grain developed (table 6). The 2,4-dichlorophenoxyacetic acid was much less effective in herbicidal action.

Discussion

Although largely without effect upon broadleaf species, isopropylphenylcarbamate proved to be a more effective herbicide upon several cereals than some halogenated phenoxyacetic acids. The fact that isopropylphenylcarbamate was not effective on *Setaria italica* may indicate that its herbicidal properties are not general for the grasses and for related families. However, the results indicate a possible avenue of approach to the control of weedy grasses, for which purpose the phenoxyacetic acid series of compounds has not been promising.

Application of isopropylphenylcarbamate via the soil was more successful in the present experiments than was its application in oil-sprays to aerial plant

TABLE 6

THE EFFECT OF ISOPROPYLPHENYLCARBAMATE AND 2,4-DICHLOROPHENOXYACETIC ACID APPLIED IN TRIBUTYLPHOSPHATE-OIL SPRAY TO WINTER RYE IN THE PREBOOTING STAGE OF DEVELOPMENT. YIELD OF GRAIN IN GRAMS WAS TAKEN AT MATURITY

Treatment*	Rate (pounds per acre)	Yield of grain in grams				
		Replications				Total
		1	2	3	4	
Isopropylphenylcarbamate	2	154	94	161	132	541
Isopropylphenylcarbamate	10	0	0	0	0	0
2,4-dichlorophenoxyacetic acid	2	95	136	216	124	571
2,4-dichlorophenoxyacetic acid	10	70	82	67	105	324

* The untreated control plots were lost during the threshing operation.

The results of several oil-spray experiments in the greenhouse were inconclusive. Rates of isopropylphenylcarbamate up to 0.5 gm. per square yard, applied in 10 ml. of tributylphosphate-oil per square yard, did not inhibit vegetative growth of young oats, wheat, rice, barley, corn, or millet to a statistically significant degree. Indications were obtained that similar applications to oats in the prebooting stage of development greatly reduced grain yield.

Isopropylphenylcarbamate was compared with 2,4-dichlorophenoxyacetic acid in a field experiment with winter rye. These compounds were applied at a

parts. The high effectiveness of oil-spray applications upon winter rye indicates that the spray method of application may be useful under field conditions.

Further studies of isopropylphenylcarbamate and similar compounds, as well as methods of application, are in progress.

Summary

1. Comparisons have been made of the herbicidal effectiveness of isopropylphenylcarbamate and some phenoxyacetic acid derivatives.

2. While isopropylphenylcarbamate applied in aqueous solution to soil at rates of $1\frac{1}{2}$–6 mg. per 4-inch pot severely stunted or killed seedling oats, wheat, corn, barley, and nonflooded rice, similar applications of the phenoxyacetic acid derivatives did not cause statistically significant inhibition of growth.

3. Isopropylphenylcarbamate applied to the soil was ineffective in herbicidal activity upon soybeans, kidney beans, cowpeas, sunflowers, radishes, turnips, and sugar beets. It possibly had a slight stimulatory action on tomatoes and appeared to cause stunting of young potatoes, an effect which was subsequently outgrown. The germination of buckwheat was completely prevented by isopropylphenylcarbamate.

4. Isopropylphenylcarbamate proved to be a highly effective herbicide upon field-grown oats and rye when applied in sawdust to the soil at seeding time or to seedling plants. It had a lesser inhibiting action upon older plants.

5. The results of applications of isopropylphenylcarbamate in oil sprays were inconclusive. Although the vegetative growth of greenhouse cereals was not inhibited, field-grown winter rye was killed.

6. Isopropylphenylcarbamate is a highly selective herbicide for certain cereals, but whether its toxicity is general for the Graminae and related families is not known. The possible use of this compound as a herbicide for the control of weedy grasses is suggested.

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OBSERVATIONS ON THE GROWTH OF CERTAIN PLANTS IN NUTRIENT SOLUTIONS CONTAINING SYNTHETIC GROWTH-REGULATING SUBSTANCES

I. SOME EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID[1]

D. L. TAYLOR, MAJ., A.U.S.

Introduction

Attention has been directed toward the possible herbicidal use of certain substances known to possess regulatory activity affecting the amount and type of growth. Such use was suggested as early as 1941 by E. J. KRAUS. Since then, similar suggestions have been made independently in the literature by a number of authors (**1, 2, 5, 6, 10, 11**). These and more recent reports (**3, 12, 14, 15, 18**) have centered attention especially on chlorinated derivatives of phenoxyacetic acid and on naphthoxyacetic acid. The susceptibility of young broadleaf plants to herbicidal treatments with growth-regulators appears, in general, to be high. Cereal crops are affected, but they appear only slightly so at most levels of treatment that are inhibitory or toxic to broadleaf plants.

Herbicidal effects have resulted when these chemicals have been applied as sprays at a rate of the order of 1 pound per acre or as soil applications, with some solid inert diluent to obtain an active-ingredient rate of the order of 2 pounds per acre. The latter method is highly interesting when considered with respect to common farming equipment and practices. Accordingly, it seemed desirable to attempt to investigate further the specific effects of some such chemical regulators on some common broadleaf and cereal plants. Such tests were conducted with young vegetative plants during the third through the sixth weeks of growth or some part of that period, unless otherwise specified.

The nutrient-solution culture technique was selected as one means of investigation in view of the possibility that growth-regulators might be employed as soil (root) treatments and because this technique allows accurate observation of the responses of root systems, as well as shoot responses. It was also possible by means of this technique to conduct tests dealing with treatment presentation time and to obtain some information about the modifying influence of soil on the effectiveness of known amounts of chemical growth-regulator. A number of investigators have previously used the nutrient-solution technique to investigate some of the effects of growth-regulating substances in relation to nutrition and growth of kidney beans and ornamental plants (**4, 9, 17**).

Methods and materials

Large numbers of Silver King (dent) corn, Illini soybeans, Marquis wheat, Victory oats, Onsen rice, and red kidney bean seeds, which were selected for soundness and uniformity, were planted in the greenhouse in flats containing gravel which was kept moist with HOAGLAND'S standard nutrient solution (**7**). When seedlings had attained the desired size, they were transferred to 6700-ml. glazed-clay nutrient-solution containers

[1] These studies were conducted at Camp Detrick, Frederick, Md., from September through December, 1944, under the supervision of Dr. A. G. Norman.

and grown for 1 week to allow seedlings to become established before treatment. All endosperm or seed residues were removed from the cereal seedlings at the time of their transfer into solution cultures.

The standard nutrient solution used in these tests (except in the test on rice) contained chemically pure chemicals in the following concentrations: 0.005 M. monopotassium acid phosphate, 0.001 M. ammonium dihydrogen phosphate, 0.005 M. calcium nitrate, 0.005 M magnesium sulphate, 0.00012 M. ferric citrate, and minor elements in the amounts recommended by HOAGLAND (7). The nutrient solution was prepared with distilled water, and the pH of the solution was adjusted initially to 6.0–6.2 with sodium hydroxide. All solutions and treatments were renewed weekly, except where different management in a test is otherwise specified.

It was normal procedure to aerate the cultures for at least 1 hour daily, excepting rice cultures, which were not aerated. A minimum continuous photoperiod of 12 hours was maintained during the winter months by means of a line of five 150-watt Mazda lamps equipped with 14-inch reflectors, suspended at intervals of 4 feet along the center of the 19×3.5-foot bench on which the cultures were located in randomized blocks.

Experimental results

EXPERIMENT I

In preliminary observational tests, 10-day-old soybean and kidney bean plants were exposed for 2 weeks to nutrient solutions containing 0.04–3.3 p.p.m. of 2,4-dichlorophenoxyacetic acid. Cowpeas were treated with 0.08–0.6 p.p.m. of this growth regulator. Treated plants developed no uniform or distinct symptoms of stem curvature or epinasty. By the 9th day it was apparent that shoot growth of all plants was decreased with 0.08 p.p.m., while growth of axillary buds from the cotyledonary nodes was stimulated by 0.04 p.p.m. After the 10th day of treatment, soybean and kidney bean plants

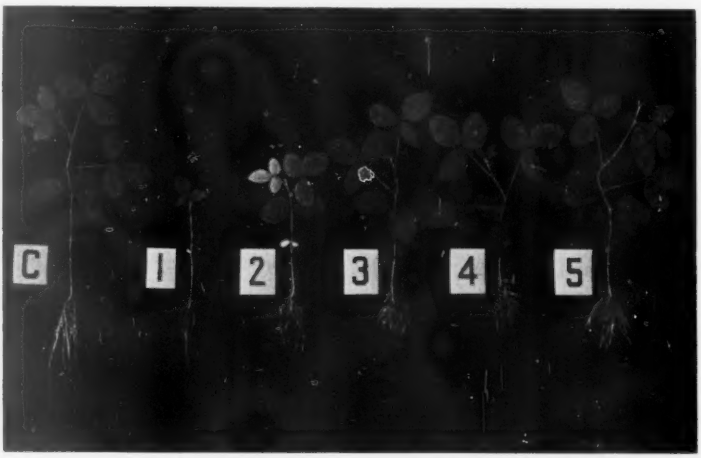


FIG. 1.—Soybean plants grown for 9 days in nutrient solution (*C*), and in similar solution containing 2,4-dichlorophenoxyacetic acid at the following concentrations: *1*, 0.66; *2*, 0.33; *3*, 0.16; *4*, 0.08; and *5*, 0.04 p.p.m.

were permanently wilted in cultures which contained 0.6 p.p.m. or more. Though shoots of cowpea plants did not wilt during 2 weeks of continuous exposure, their appearance was distinctly chlorotic and unhealthy in cultures which contained 0.3 p.p.m. or more of 2,4-dichlorophenoxyacetic acid. The increase in dry weight of root, shoot, or total plant was inversely related to concentrations of growth-regulator. The growth of plants treated with 0.08 or 0.16 p.p.m. was reduced to approximately 75 and 50 per cent, respectively, of that of untreated plants (fig. 1).

In cultures which contained different volumes of solution (1700, 3600, and 6700 ml.) and equal weights of 2,4-dichlorophenoxyacetic acid, the amount of cowpea growth which occurred during a 2-week period suggested that the effect of a given amount of the regulator was influenced by the concentration presented (fig. 2). Representative plants are shown in figure 3. Consistently, the earliest and most outstanding symptom which appeared on treated plants was swelling of the lower hypocotyl and parts of roots. This was evident on the 3d day in solutions containing more than 0.04 p.p.m. and became more marked until, by the 7th day, the surfaces of hypocotyls of kidney beans were corky and

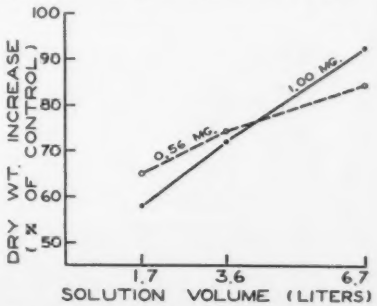


FIG. 2.—The influence of concentration on the effect of 2,4-dichlorophenoxyacetic acid on the growth of cowpeas. Labels on curves indicate weight of regulator per culture vessel. The final dry weight of untreated plants is valued as 100 per cent.

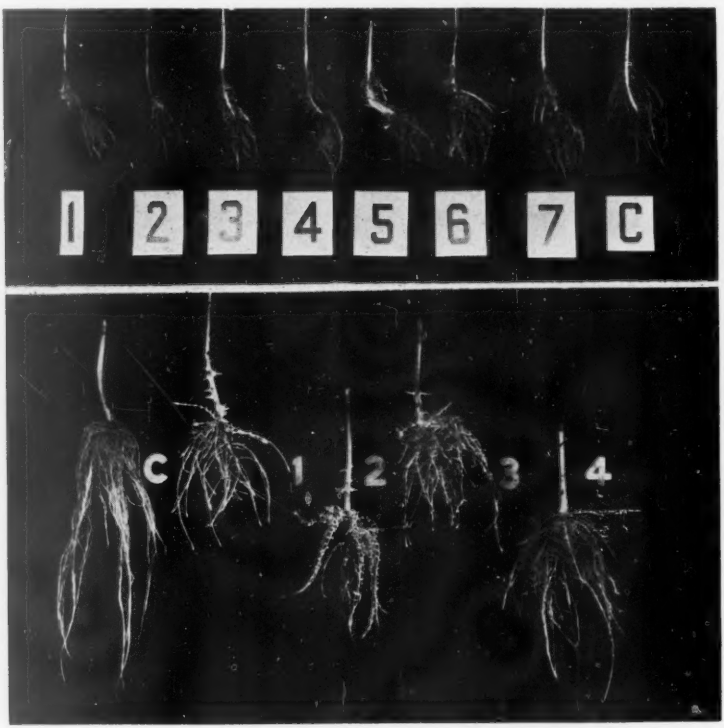
FIG. 3.—The influence of concentration and amount of 2,4-dichlorophenoxyacetic acid on growth of cowpeas during a 2-week test period.

TREATMENTS

Number	5	3	1	C	2	4	6
Regulator (mg.)	0.55	1.0	2.0	0.0	2.0	1.0	0.55
Culture volume (liters)	6.9	6.9	6.9	3.6	3.6	3.6	3.6
Regulator (p.p.m.)	0.08	0.15	0.29	0.0	0.56	0.28	0.15

split by longitudinal fissures in all concentrations of 0.6 p.p.m. or less. In respective order, soybeans and cowpeas showed this response in lesser degrees.

Production of thick, non-elongated roots from the lower hypocotyl of kidney beans and, to lesser degree, in soybeans and cowpeas was stimulated by 0.04 p.p.m. or more. Such roots on kidney beans often became fasciated. These characteristics became more striking as the concentration was increased from 0.16 to 0.6 p.p.m. Adventitious roots or fasciated sheets of tissue were oriented mainly in four radial planes along the hypocotyl, the general appearance of which was similar to that previously noted when various growth-regulators were applied to shoots of plants (**8, 15**) (figs. 4 and 5). Growth was so completely arrested by more than 0.6 p.p.m. of 2,4-dichlorophenoxyacetic acid that these symptoms were expressed in only a slight degree prior to the death of the plants.



FIGS. 4, 5.—Fig. 4, the effect of 2,4-dichlorophenoxyacetic acid on hypocotyls and roots of kidney bean plants. Treatments: *1*, 3.30; *2*, 1.70; *3*, 0.60; *4*, 0.33; *5*, 0.16; *6*, 0.08; *7*, 0.04 p.p.m. of the regulator; *C*, control in nutrient solution only. Fig. 5, hypocotyls and root systems of cowpea plants grown in nutrient solutions containing 2,4-dichlorophenoxyacetic acid. Treatments: *1*, 0.60; *2*, 0.33; *3*, 0.16; *4*, 0.08 p.p.m. of the regulator; and *C*, control in nutrient solution only. Decreased root elongation in *4*, decreased elongation and swelling of root tips in *3*, marked swelling of lateral roots in *2*, general lack of growth in *1*, and progressive decrease of adventitious root growth from hypocotyls in *1–4*.

Root systems of plants grown in

treated solutions were somewhat darkened, possibly because of tissue deterioration or death. Elongation of primary and lateral roots, almost lacking at 0.33 p.p.m., was inhibited in proportion to regulator concentration. Distinct suppression of root elongation, accompanied by thickening, especially in the region 2–5 mm. behind root tips, was evident on plants treated for 4 days with 0.16 p.p.m., and by the 12th day in 0.04–0.08 p.p.m.

Experiment II

In a second test on 17-day-old soybeans, triplicate cultures, each of which contained four plants, were treated with 2,4-dichlorophenoxyacetic acid from 1 to 60 p.p.m. The time required to kill plants was inversely related to the concentration of regulator in the nutrient (fig. 6). Death of more than one-half of the plants in cultures containing 1 p.p.m. and of all plants in cultures with more than 2 p.p.m. had occurred by the end of the 8th day of treatment. Untreated plants were growing normally when the test ended on the 10th day. For any one concentration the time required to kill all plants in the triplicate cultures varied not more than 1 day from the average time indicated for that concentration.

During the test period, true stem curvatures or epinastic responses occurred in a small proportion of the treated plants. The frequency of this response was erratic and was not clearly relatable to the concentration applied. The most typical symptom, namely, general wilting of leaves and shoots, appeared after periods of treatment which varied inversely with the concentration. The foliage was strongly wilted after 18 hours in nutrient solutions containing 25 p.p.m. or more. The primary leaves wilted first, then wilting rapidly progressed upward to involve all leaves and the entire shoot.

As soon as all plants in any culture were dead, shoots of the plants were divided into two fractions—new shoot and old shoot or the shoot up to the primary leaf node plus the primary leaves—and dry-weight data were obtained (table 1). Although some plants at the lower concentrations did not die for a number of

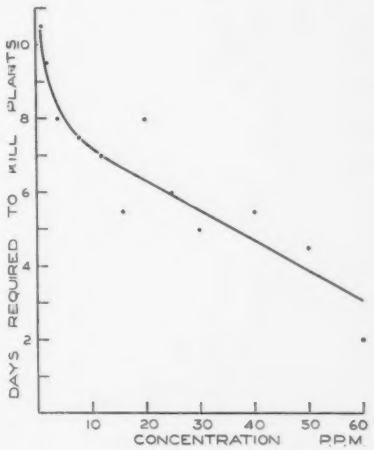


Fig. 6.—Length of time to death after addition of 2,4-dichlorophenoxyacetic acid to 17-day-old soybean plants in nutrient solution. Time shown is average for triplicate cultures, each having four plants.

days, the weight data indicate that significant increase in plant dry weight did not occur in concentrations above 2 p.p.m. Irregular growth in cultures containing 1 or 2 p.p.m. resulted in plant dry-weight increases of 10 per cent compared to control plants. There was no indication of increase in dry weight of roots with more than 2 p.p.m. The 50 per cent increase in weight of roots in 1 p.p.m. was not due to normal development. In cultures which contained 4 p.p.m. or less, any visible new shoot growth, which appeared before the death of plants, was

much stunted and irregular. The major increase in plant weight appeared attributable to somewhat irregular development of hypocotyls, which occurred on plants grown in concentrations as high as 25 p.p.m. Growth of old portions of the shoot, new shoot, and roots, respectively, were increasingly inhibited by 2,4-dichlorophenoxyacetic acid.

No visible symptoms had appeared in 48 hours after the plants were initially placed in 1 p.p.m., but the lower leaves of some plants in 5 p.p.m. had developed a slightly lighter color than those of untreated plants, and distinct stem curvature was evident near the shoot apex. These responses were distinct on the 3d day, when the lower leaves of some

TABLE 1

THE INCREASE IN DRY WEIGHT OF 17-DAY-OLD SOYBEAN PLANTS AFTER ADDITION OF 2,4-DICHLOROPHENOXYACETIC ACID TO THE NUTRIENT SOLUTION. MEAN WEIGHTS SHOWN REPRESENT FOUR PLANTS

REGULATOR (P.P.M.)	FINAL DRY WEIGHT (MG.)*				PERCENT INCREASE IN WEIGHT†			
	Old shoot	New shoot	Roots	Total plant	Old shoot	New shoot	Roots	Total plant
0	720 ± 60	2330 ± 300	370 ± 60	3420 ± 520	100	100.0	100	100.0
1	470 ± 90	390 ± 30	240 ± 70	1100 ± 200	20	6.0	46	11.0
2	500 ± 120	380 ± 10	160 ± 60	1040 ± 200	29	5.5	12	9.0
4	460 ± 50	370 ± 40	90 ± 20	920 ± 100	16	5.0	0	4.0
8	460 ± 30	340 ± 20	70 ± 10	880 ± 20	16	3.5	0	2.5
12	490 ± 40	350 ± 20	90 ± 20	930 ± 80	26	4.0	0	4.5
16	430 ± 30	290 ± 70	70 ± 10	790 ± 70	7	1.0	0	0.0
20	480 ± 60	270 ± 50	90 ± 10	830 ± 140	23	0.0	0	1.0
25	470 ± 50	310 ± 50	70 ± 10	830 ± 120	19	2.0	0	1.0
30	420 ± 50	200 ± 20	70 ± 10	700 ± 70	3	0.0	0	0.0
40	380 ± 40	240 ± 30	60 ± 10	690 ± 60	0	0.0	0	0.0
50	370 ± 10	260 ± 20	100 ± 20	730 ± 40	0	0.0	0	0.0
60	410 ± 20	330 ± 10	110 ± 10	850 ± 50	0	3.0	0	1.5
Initial weight (mg.)	410	270	130	810	.....	.....	.....	.....

* Mean dry weight and maximum variation are shown.

† The increase in dry weight of untreated plants or plant parts is valued as 100 per cent.

EXPERIMENT III

Three-week-old kidney beans, which had two trifoliate leaves unfolded on shoots 8–10 inches long above the primary leaf node, were exposed for 2 and 3 days to 1 and 5 p.p.m. of 2,4-dichlorophenoxyacetic acid. After the desired presentation time had elapsed, the plants were removed from each of the duplicate cultures and placed in normal nutrient solution. Observations of symptoms and responses were made during the succeeding 14 days.

plants which had remained in 1 p.p.m. were chlorotic. At this time the older leaves on plants in 5 p.p.m. were distinctly wilted.

During the period from the 3d through the 6th day, all treated plants appeared to make some recovery in the plain solutions. All lower leaves of plants treated with 1 p.p.m., especially those exposed only 2 days, became more green. Though the lower leaves of plants treated with 5 p.p.m. were dry or dead on the 6th day, the shoots had recovered from the curva-

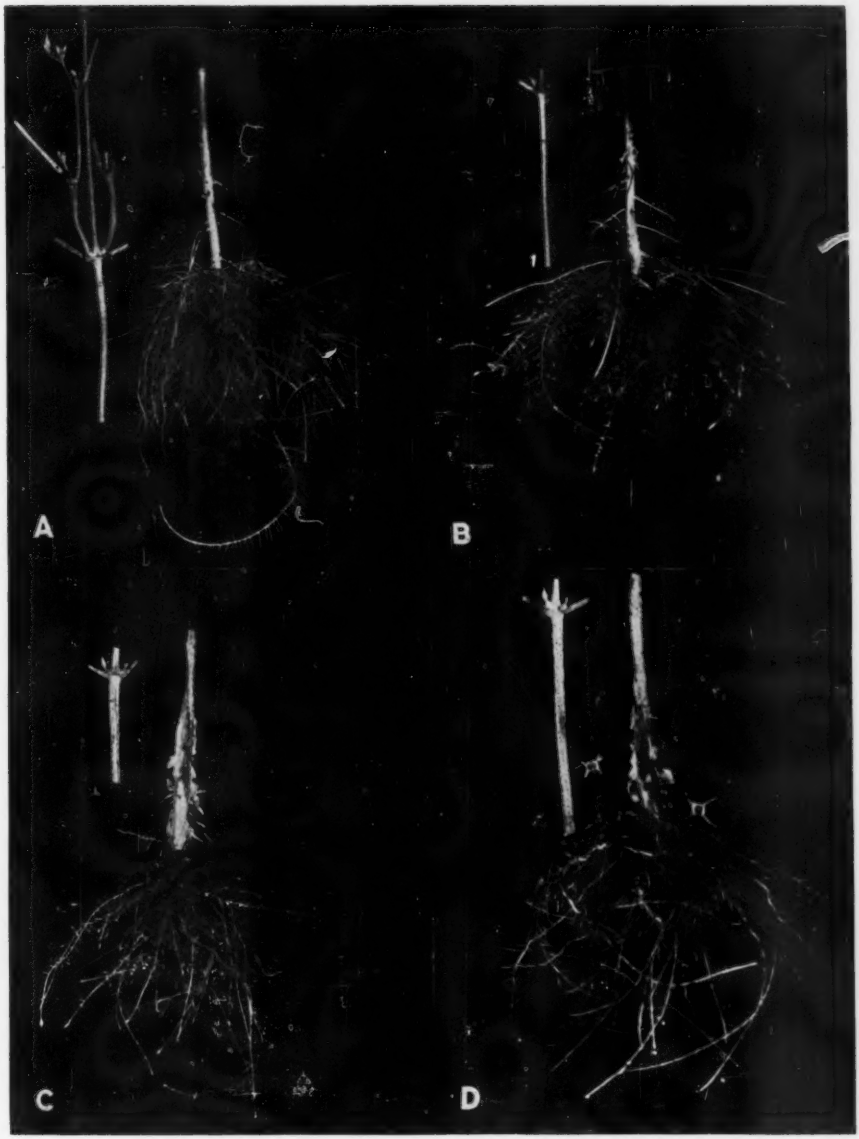


FIG. 7.—Root systems, hypocotyls, and lower shoots of kidney bean plants variously treated with 2,4-dichlorophenoxyacetic acid in nutrient solutions. Treatments initiated on the 15th day of growth and plants photographed 15 days later. *A*, plant not treated; *B*, 2 days of exposure to 1 p.p.m.; *C*, 2 days of exposure to 5 p.p.m.; *D*, 3 days of exposure to 5 p.p.m. Progressive increase in inhibition of elongation and branching of roots; increase in swelling of hypocotyl and production of adventitious roots as treatment intensity was increased; maximum root-tip swelling on plants exposed for 2 days; and axillary bud proliferation at 5-p.p.m. treatment.

ture response. Hypocotyls were swollen and the meristem regions of roots and shoots were slightly swollen.

In 8–10 days after initial application of 3-day treatments with 5 p.p.m. of 2,4-dichlorophenoxyacetic acid, the hypocotyls were much swollen, and terminal, and some lateral shoot meristems were "barrel-like" hypertrophies, tipped by stunted, mottled, dark-green leaves. These symptoms developed less strongly in plants treated with 1 p.p.m. or for 2 days with 5 p.p.m. In proportion to the intensity (concentration × time) of regulator treatment applied, production of adventitious roots from the swollen lower hypocotyl region was greatly stimulated. Three days of exposure to 5 p.p.m. resulted in fasciation of such roots to form four vanes or sheets of tissue extending along and radially out from the ruptured hypocotyls. These symptoms and typical shoot-system responses are shown in figures 7 and 8.

At the end of the test period the amount of growth of shoots of plants exposed for 1 day to 1 p.p.m. appeared equal to that of control plants. Relatively little growth of shoots had occurred in plants treated 3 days with 5 p.p.m., and intermediate treatments caused visible inhibition. Untreated plants were flowering profusely at the end of the test period, whereas the 3-day treatment with 5 p.p.m. delayed or completely curtailed reproductive development. All treat-

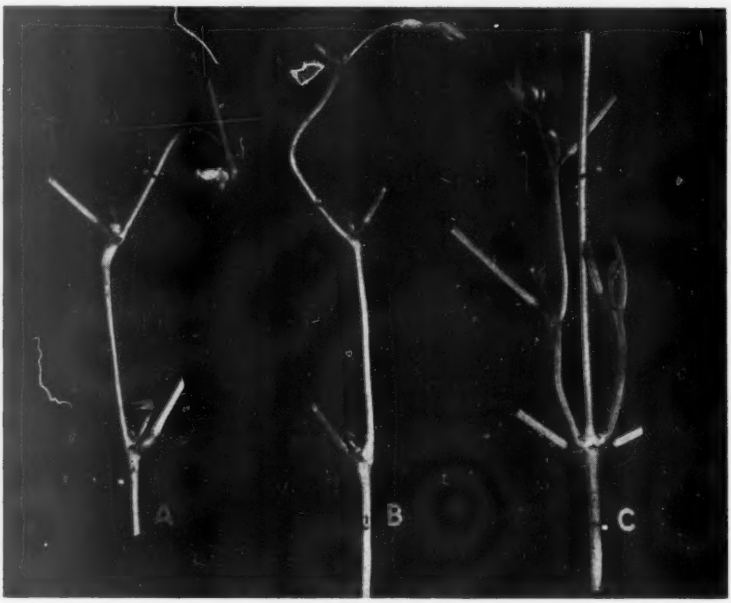


FIG. 8.—Upper shoots of kidney bean plants variously treated with 2,4-dichlorophenoxyacetic acid, 15 days after initiation of treatments. *A*, 3-day exposure to 5 p.p.m.; *B*, 2-day exposure to 5 p.p.m.; *C*, untreated. Decreased shoot growth and etiolated internodes, suppression of normal growth, and swelling of terminal and lateral meristems as a result of treatments.

ments caused degrees of this response which were proportional to treatment intensity.

Experiment IV

Preliminary tests were conducted on wheat and corn plants in 1700-ml. cultures during the period from the 2d through the 5th weeks of growth. Wheat cultures, each containing ten plants, were treated with concentrations of from 3 to 8 p.p.m. Concentrations ranging from 0.5 to 8.0 p.p.m. were applied to triplicate corn cultures, each containing three plants. At the start of the test period the shoots of wheat plants were 6 inches long; and those of corn were 8–10 inches in length, with the fourth leaf just visible.

During the test periods no sign of any shoot curvature or growth response of leaves of these plants was observed. Though both corn and wheat plants growing in 8 p.p.m. were alive after 1 week of treatment, control plants were visibly further advanced than all plants treated with more than 0.5 p.p.m. Symptoms were evident on roots of treated plants by the 4th day (figs. 9 and 10). Representative wheat cultures treated for 20 days are shown in figure 11.

TABLE 2

Final dry weight of wheat and corn plants grown in nutrient solutions containing 2,4-dichlorophenoxyacetic acid. Average weights shown are for triplicate corn and duplicate wheat cultures treated continuously from the 2d through the 5th weeks of growth

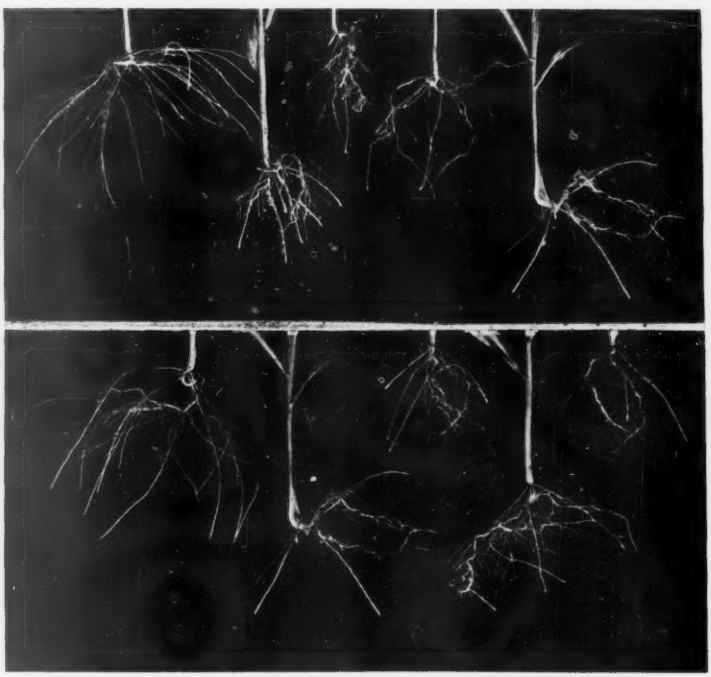
Regulator (p.p.m.)	Weight per corn plant						Weight per wheat plant	
	Shoots		Roots		Total plant			
	Mg.	%	Mg.	%	Mg.	%	Mg.	%
0.0	160.6	100	47.6	100	208.3	100	226	100
0.5	61.6	39	31.6	67	93.2	45	.....	.....
1.0	41.0	26	25.6	54	66.6	32	.....	.....
2.0	35.3	22	23.3	49	58.6	28	.....	.....
3.0	.....	.....	.....	.....	.....	.....	87	39
4.0	36.3	23	17.6	37	53.9	26	76	34
6.0	.....	.....	.....	.....	.....	.....	61	27
8.0	33.6	21	12.6	27	46.2	22	51	23
Initial weight (mg.)	33.0	.....	8.5	.....	41.5	.....	.....	.....
	Minimum significant differences—mg.							
At 99:1 odds	23.8		24.1		9.7		.....	
At 19:1 odds	17.1		17.3		7.0		33	

At the end of the test period, normal development of roots had been almost completely inhibited by 4 p.p.m. or more of the regulator. The symptoms which developed on roots of these plants were similar to those observed on broadleaf crops with regard to type and time of expression. Crowns of wheat plants were distinctly swollen in cultures containing 3–6 p.p.m. Maximum abnormal development of this type appeared to occur at

less than 6 p.p.m., possibly because higher concentrations almost completely arrested all growth (fig. 12). Stimulation of the growth of adventitious roots from the crowns of treated corn plants was less evident than for wheat plants.

parts of the youngest leaves remained green at 4 p.p.m.

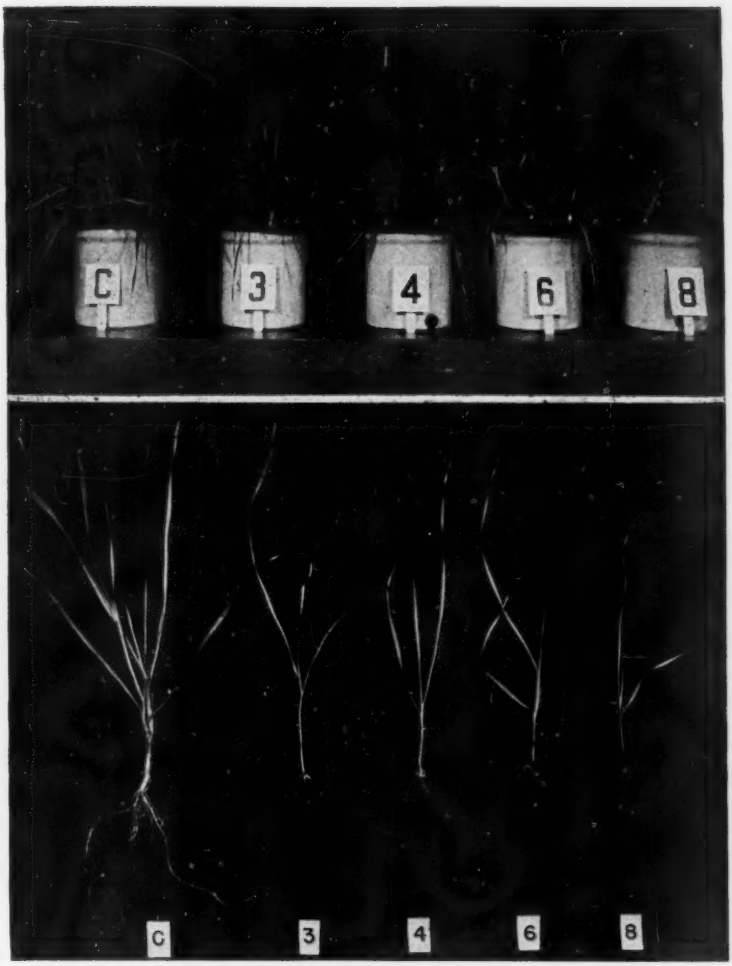
The lowest 2,4-dichlorophenoxyacetic acid concentrations tested, namely, 3 and 0.5 p.p.m. on wheat and corn respectively, resulted in highly significant re-



FIGS. 9, 10.—Fig. 9, roots of 26-day-old corn plants after 4 days of growth in nutrient solution containing (left to right) 0.0, 0.5, 1.0, 2.0, and 4.0 p.p.m. of 2,4-dichlorophenoxyacetic acid. Marked inhibition of growth caused by more than 2 p.p.m.; increased inhibition as concentration increased; root tips swollen; and general lack of development or stimulation of adventitious roots. Fig. 10, root systems of corn plants after 4 days in nutrient solution containing (left to right) 0.0, 4.0, 8.0, 15.0, and 25.0 p.p.m. of 2,4-dichlorophenoxyacetic acid.

The growth of shoots of both crops was markedly less in all concentrations tested. After the first week of the test period, shoots did not appear to increase in length, and, progressing upward on the shoot, leaves dried from the tip toward the base. At the end of the test, leaves of plants treated with 6 p.p.m. or more were completely dead, and only

ductions of 60 and 55 per cent in the dry weight of the plants (table 2). Increase in the concentration of the regulator to 8 or to 1 p.p.m. or more, respectively, resulted in significantly less average final dry weight of wheat and corn plants. Compared to untreated plants, the final dry weight of wheat plants grown in 6–8 p.p.m. was 25 per cent, while an ap-



FIGS. 11, 12.—Fig. 11, shoots of wheat plants in cultures containing 3–8 p.p.m. of 2,4-dichlorophenoxyacetic acid after 20 days of continuous treatment. Numbers on pots indicate p.p.m. concentrations. Shoots are partly dry in the 3-p.p.m. culture, and in other treated cultures plants are dead. Fig. 12, representative wheat plants after 20 days of growth in cultures which contained 0, 3, 4, 6, and 8 p.p.m. of 2,4-dichlorophenoxyacetic acid. Inhibition of growth in proportion to concentration; marked swelling at the crown region; some increase of adventitious root growth, which was soon arrested; treated roots dark in color.

proximately equal degree of reduction of weight of corn plants occurred in 4 p.p.m.

The roots of corn plants continued to increase in dry weight in solutions containing more than 1 p.p.m., although shoot growth was completely arrested (fig. 13). In normal plants the weight of shoots accounted for the major portion of the total increase in plant weight, whereas the major part of the increase in weight of corn plants grown in more than 0.5 p.p.m. was due to growth of roots.

Judging from visual observation, this relation did not appear to hold with

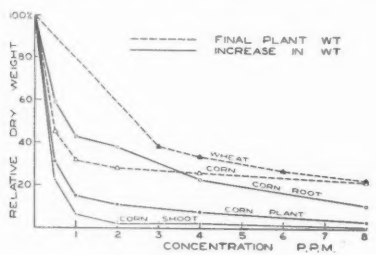


FIG. 13.—The increase in dry weight of wheat and corn plants during the 2d through the 5th weeks of growth in nutrient solution containing 2,4-dichlorophenoxyacetic acid.

treated wheat plants. The fact that growth of shoots of corn did not parallel growth of roots prior to death of plants, as well as the appearance of the root systems, suggests that the root tissues which did develop were abnormal and did not function normally.

Discussion

Employing sand cultures, LAUDE (9) found that treatment with low concentrations of indoleacetic acid resulted in an increase in the weight of kidney bean plants when potassium in the nutrient solution did not limit growth. The roots of plants were more fibrous and increased approximately 50 per cent over normal weight when similar concentrations of naphthalene acetamide were applied in the nutrient solution, which responses confirmed earlier observations by other investigators (13). At this concentration the growth of shoots was significantly reduced. Using a comparable technique with marigolds, SWARTZ (17) found that 0.01 mg. of naphthalene acetic acid per liter of nutrient solution decreased the weight of shoots and of the total plant, but only slightly decreased the weight of roots. Such treatments did not inhibit cosmos plants, and 0.1 mg. per liter did not result in significant change in the dry weight of chrysanthemums. Visible effects on the growth habit of the plants or on floral initiation were lacking.

In the present investigations, when 0.5–1.0 p.p.m. of 2,4-dichlorophenoxyacetic acid was supplied in solution, the increase in the dry weight of all parts of soybeans was significantly reduced, and the habit of the plants was changed. It was indicated in these tests, and confirmed in more detailed studies to be described in later papers of this series, that less inhibition of increase resulted in the weight of root than of shoot or total plant when several plants were treated with 2,4-dichlorophenoxyacetic acid or similar substituted phenoxyacetic acid derivatives. Floral initiation in kidney beans was retarded by low-intensity treatments (for 2 or 3 days at 1 or 5 p.p.m.) with this regulator.

Morphological changes of parts of shoots and roots of crop plants did result from treatment with 2,4-dichlorophenoxyacetic acid. Continuous treatments with 0.25–60 p.p.m. of this acid appeared to stimulate such responses in shoots less than milder discontinuous treatments. Curvatures of stems and formation of shoot meristem hypertrophies were infrequent in these tests, and these occurred erratically with respect to treat-

ment concentration. The most consistent response of shoots was complete cessation of growth, which, in proportion to the concentration of regulator applied, was soon followed by wilting and drying of parts of plants. Though swelling of the crown region of cereals was distinct, in response to equivalent treatments relatively more obvious changes occurred in the type of growth of shoots of the broadleaf crops.

Morphological changes in the root systems of both groups of plants were the most striking and consistent responses which developed. In this respect and also in regard to the relative amount of growth of roots, the results in these tests with 2,4-dichlorophenoxyacetic acid most nearly correspond to those described by HAMNER (4), who used alpha-naphthalene acetamide on kidney beans in nutrient sand cultures. In addition to decreasing the amount of growth of shoots and inhibiting expansion of leaves, which became curled and thickened, such treatment with 0.34 mg. of the latter regulator per liter resulted in short, stubby roots of approximately twice normal diameter, the increases in the weight of which were up to 37 per cent more than normal. Production of secondary roots occurred from about 0.6 mm. back from the apical meristem, where xylem and phloem elements were found to have matured early. Such responses were not described for treatments with phenylacetic acid, which consistently decreased the dry weight of bean plants and caused greater reduction in weight of shoots than of roots.

The much-inhibited, nonfibrous, nonelongated, discolored, bulbous-tipped root systems, with abnormal numbers of adventitious roots, which were developed when species of both groups of plants were treated with 2,4-dichlorophenoxyacetic acid in these tests, much resembled those of beans grown in alpha-naphthalene acetamide. At the same time, their dry weight was much decreased from normal. In contrast to increased weight of roots caused by the latter regulator and the lack of marked effect of treatment with phenylacetic acid, 2,4-dichlorophenoxyacetic acid treatments significantly reduced the dry weight of the root systems of all species tested. The morphological responses of these plants to treatments with 2,4-dichlorophenoxyacetic acid very much resembled the telemorphic responses of roots of sweet peas which were observed to follow treatment of the shoots with 4-chlorophenoxyacetic acid (1).

The growth of cereals, though relatively less affected than that of broadleaf crops (19), was somewhat modified in form and distinctly reduced by treatment with low concentrations of 2,4-dichlorophenoxyacetic acid. This was interesting, considering the emphasis in the literature on the differential effect of this compound. The results of these tests and of other more extensive tests, in which similar responses occurred, to be reported in later papers of this series, in part parallel those recently reported concerning the growth of several lawn grasses (12). Detrimental effects of the acid on these grasses were shown to be temporary in the soil and to last not more than 5 weeks after treatments up to 3 pounds per acre had been applied. Though growth was significantly stunted for periods of from 5 to 14 weeks following the applications, depending on the rate of treatment, established plants were not killed by $\frac{3}{4}$–3 pounds per acre of 2,4-dichlorophenoxyacetic acid. Except with the most rigorous treatments, they recovered, to make normal growth later.

Like lawn grasses, the cereal crops re-

mained alive at the end of test periods in nutrient solutions containing amounts of regulator which during equal or shorter periods proved lethal to the broadleaved crops. Distinct differential effects could result if treatment were temporary, allowing the more resistant cereals to recover later and to continue normal growth.

Summary

Young vegetative red kidney bean, soybean, cowpea, wheat, and corn plants were grown for from 2 to 3 weeks in nutrient-solution cultures which contained various concentrations of 2,4-dichlorophenoxyacetic acid.

This growth-regulator, in proportion to the concentration supplied, proved toxic to all species tested, reducing or inhibiting growth and causing distinct morphological changes in these plants.

Cereal crops were slightly more resistant than broadleaved crops. The growth of broadleaved plants was decreased by concentrations as low as 0.15 p.p.m., and soybeans were dead after exposure to from 1 to 5 p.p.m. for from 8 to 10 days. Cereal plants were alive after 20 days in nutrient solutions containing 3 p.p.m. or more, though their dry weight was significantly decreased by the lowest concentrations in which they were grown, namely, 0.5 and 3.0 p.p.m. The broadleaved plants appeared to have resistance to the regulator, increasing slightly in the following order: kidney bean, soybean, and cowpea. Wheat appeared more resistant than corn.

The dry weight of the shoots of plants was decreased slightly more than that of roots by low concentrations, but the modification of the habit of root systems was more consistent and distinct.

In proportion to concentration, 2,4-dichlorophenoxyacetic acid decreased elongation and branching of roots, caused swelling of root tips, swelling of some shoot meristems, and thickening of the hypocotyls of broadleaved plants, from which the growth of adventitious roots was stimulated by low concentrations. Few epinastic or stem-curvature responses occurred on shoots, which more typically wilted. The morphological responses of the shoots of broadleaved plants were more striking than those of cereals.

Wilting and drying of leaves and swelling of the crown region, from which the growth of adventitious roots was stimulated, were noted on shoots of cereals, the root systems of which responded quite similarly to those of broadleaved plants.

Low-intensity treatments retarded floral initiation of red kidney beans.

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OBSERVATIONS ON THE GROWTH OF CERTAIN PLANTS IN NUTRIENT SOLUTIONS CONTAINING SYNTHETIC GROWTH-REGULATING SUBSTANCES

II. THE INFLUENCE OF PRESENTATION TIME[1]

D. L. TAYLOR, MAJ., A.U.S.

Introduction

In preliminary tests (2) it was observed that, when low concentrations of 2,4-dichlorophenoxyacetic acid were added to nutrient solutions in which species of broadleaf and cereal crops were developing, growth was arrested, or marked morphological changes ensued, especially in the root systems. In several quantitative tests, plants were exposed to 2,4-dichlorophenoxyacetic acid for various periods of time for comparison with continuous treatment with that compound. After such exposure they were removed to cultures free of growth-regulator, and observations of their development were continued. Such tests in which plants were subjected to known concentrations of growth-regulators for limited periods of time made possible more precise study of the effects of treatment intensity (concentration × presentation time).

The materials and methods employed in conducting these presentation-time studies were similar to those used in the preliminary tests (2).

Experimental results

Soybean plants, 2 weeks old, were treated for 1, 2, or 7 days with 1, 5, or 20 p.p.m. of 2,4-dichlorophenoxyacetic

[1] These studies were conducted at Camp Detrick, Frederick, Md., during January and February, 1945, under the supervision of Dr. A. G. Norman. The author gratefully acknowledges the able assistance given him by I. E. Freeman, Ph.M. 2/c, U.S.N.R., in conducting these investigations.

acid, after which they were transferred to normal nutrient solutions until the end of the 5th week. Similar plants were also treated continuously with each of these concentrations of the regulator from the 2d through the 5th weeks of growth.

Wilting of plants did not result from 2 days of exposure to 5 p.p.m. or less, and only slight curvature of the stems of some plants occurred. Plants in 20 p.p.m. were wilted by the second day, and there was moderate curvature of stems of some of this group. Such wilted plants recovered turgidity in 1 day if transferred to normal nutrient solution. The leaves, especially the older ones, became chlorotic when plants were exposed for 7 days or more to 5 p.p.m. or higher concentrations. These plants did not regain normal color after transfer to plain nutrient solutions, and drying or death of such leaves progressed upward on the plants.

No shoot proliferations were noted on any of the treated plants. Typical swelling of the base of the hypocotyl and of root tips resulted from treatments of 7, 2, and less than 2 days' duration with 1, 5, or 20 p.p.m., respectively. Though some swelling was caused by longer exposure to the higher concentrations, these responses were almost lacking in 20 p.p.m. and were much reduced in 5 p.p.m. The higher-intensity treatments

TABLE 1

THE INCREASE IN DRY WEIGHT OF SOYBEAN PLANTS WHICH OCCURRED DURING THE 3D THROUGH THE 5TH WEEKS OF GROWTH WHEN 2-WEEK-OLD PLANTS WERE EXPOSED FOR 1, 2, OR 7 DAYS OR CONTINUOUSLY TO CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID IN NUTRIENT SOLUTIONS. MEAN WEIGHTS ARE SHOWN FOR FOUR REPLICATES, EACH CONTAINING TWO PLANTS*

Concentration (p.p.m.)	Presentation time (days)	New shoots		Roots		Total plant	
		Final weight (mg.)	Weight increase (%)	Final weight (mg.)	Weight increase (%)	Final weight† (mg.)	Weight increase (%)
0	20	560	100	150	100	1190	100
1	1	890	160	140	88	1390	125
1	2	580	104	150	100	1110	90
1	7	290	52	130	75	720	38
1	20	80	14	120	63	510	14
5	1	230	41	110	50	700	38
5	2	510	91	130	75	950	57
5	7	60	9	40	.....	320	.....
5	20	20	4	50	.....	330	.....
20	1	110	20	90	25	520	15
20	2	210	37	80	13	560	20
20	7	30	5	20	.....	280	.....
20	20	10	2	20	.....	260	.....
Initial dry weight (mg)	.....	0	.....	70	.....	400	.....

* Minimum significant difference for mean shoot weight: for experiment at 19:1 odds, 330 mg.; for regulator treatments only at 99:1 odds, 190 mg.

† Includes the weight of the old portion of the shoot.

more nearly arrested all types of development.

The average increase in the total fresh weight of untreated plants was four times the initial weight of the plants. The mean weight of new shoot (weight of shoot above the primary leaf node) comprised a greater fraction of the total increase in weight than that contributed by growth of roots. Seven days of treatment with dry weight of plants increased less than one-fourth of the normal amount in 20 p.p.m. of 2,4-dichlorophenoxyacetic acid. No increase in plant dry weight occurred when 5 (or more) p.p.m. were presented for 7 (or more) days. All treatments lasting 7 days limited the increase in dry weight of plants to approximately one-half or less of the normal increase. From this test it appears that 5 p.p.m. of 2,4-di-

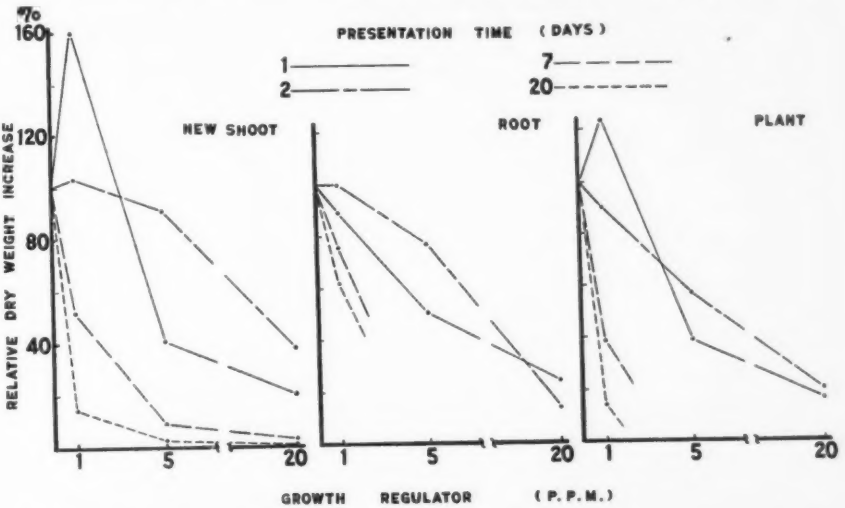

FIG. 1.—The relative increases in the dry weight of soybean plants and plant parts which occurred during the 3d through the 5th weeks of growth when 2-week-old plants were exposed to 2,4-dichlorophenoxyacetic acid in nutrient solution for 1, 2, or 7 days or continuously. The increase in weight of untreated plants, shown as 100 per cent, was 560, 80, and 790 mg. for new shoots, roots, and total plant weights, respectively.

1 or 5 p.p.m., and the 20-p.p.m. treatment for a period as short as 1 day, limited fresh-weight increases of new shoots to less than one-half of normal. Plants which grew in 5 and 20 p.p.m. for 7 days or more were dead at the end of the test period.

Dry-weight data indicate that changes either in concentration or in presentation time resulted in highly significant inhibition of soybean growth in an amount that was, in general, proportional to the treatment intensity (table 1 and fig. 1). The chlorophenoxyacetic acid presented for slightly more than 2 days will kill young soybeans or completely arrest their growth.

Only the plants treated with 1 p.p.m. for 1 day increased in dry weight more than did untreated plants. In this treatment the decrease in growth of other plant parts was more than equaled by the increase in weight due to the growth of new shoots, which was 1.6 times the normal amount. The weight of hypocotyls and old shoots decreased in high-intens-

ity treatments, while in low-intensity treatments abnormal growth of these parts resulted in subnormal dry-weight increases. The reduction of growth of roots in low-intensity treatments (1 p.p.m.; and 5 or 20 p.p.m. for 2 days ments were supplied, the symptoms developed by continuously treated plants were similar to those caused by equivalent 2,4-dichlorophenoxyacetic acid concentrations in the observational test on corn. The tips of leaves were slightly

TABLE 2

THE INCREASE IN THE DRY WEIGHT OF CORN PLANTS WHICH OCCURRED DURING THE 3D THROUGH THE 5TH WEEKS OF GROWTH WHEN 2-WEEK-OLD PLANTS WERE EXPOSED FOR 1, 2, OR 7 DAYS OR CONTINUOUSLY TO CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID IN NUTRIENT SOLUTIONS. MEANS ARE SHOWN FOR FOUR REPLICATES EACH CONTAINING TWO PLANTS*

Concentration (p.p.m.)	Presentation time (days)	Final weight and increase in weight of—					
		Shoots		Roots		Total plant	
		Mg.	%	Mg.	%	Mg.	%
0	20	1040	100	330	100	1370	100
1	1	980	93	290	85	1370	100
1	2	1010	96	370	115	1380	100
1	7	530	38	240	67	770	45
1	20	390	21	170	41	570	26
5	1	680	56	260	74	940	61
5	2	560	42	210	55	770	45
5	7	320	12	110	19	430	14
5	20	280	7	100	15	370	9
20	1	660	54	260	74	920	59
20	2	290	9	130	26	540	13
20	7	170	0	70	4	240	0
20	20	250	4	70	4	320	3
Initial dry weight (mg.)		220		60		280	

* Minimum significant differences for means of total dry weight are as follows:

	99:1 odds	19:1 odds
Presentation time	300 mg.	224 mg.
Concentration	260	194
Presentation time × concentration	520	387

or less) was, in general, less marked than that caused in shoot growth. However, the character of the root growth which occurred was distinctly abnormal.

In a similar test employing corn plants which had four visible leaves and were approximately 6 inches tall when treatwilted on plants exposed for 2 days to 20 p.p.m. and, to a lesser degree, on some plants treated with 5 p.p.m. One day following their transfer to normal nutrient solutions, most plants had recovered turgidity; by 36 hours all signs of wilting had disappeared.

Complete data on fresh weight were not obtainable because shoots of plants exposed for 7 or 20 days to more than 5 p.p.m. of 2,4-dichlorophenoxyacetic acid were partly or completely dead and dry. Roots of these plants were dark colored and watery or soft in appearance. In untreated plants the fresh weight of root, shoot, and whole plant increased in approximately similar proportions, the final weight of which was more than four times that of initial plants. Compared to untreated plants, treatment with 1 p.p.m. for 7 days or 5 p.p.m. for 2 days caused 55–60 per cent decrease in the fresh weight of plants. The fresh weight of shoots increased 30–35 per cent of normal with treatments of 20, 5, or 1 p.p.m. for 1, 2, or 7, days, respectively, but was only 15 per cent of normal when 20 p.p.m. was applied for 2 days.

It is evident from the summary of dry-weight data in table 2 and figure 2 that highly significant differences in growth inhibition, which were proportional to treatment intensity (concentration × presentation time), resulted when regulator concentration and presentation time were varied. Shoot growth was 55 per cent or more of normal only in the 1-day treatments and in the 2-day treatment with 1 p.p.m. Treatment with 5 p.p.m. for 7 days or more and with 20 p.p.m. for 2 days or more reduced increases in the dry weight of shoots by approximately 90 per cent. Again the relative increase in the weight of corn roots (due to proliferation and abnormal development) was greater than that of shoots of treated plants. Increases in dry weight of roots were limited to 25 per cent or less by 5 and 20 p.p.m. presented for 7 and 2 days, respectively.

Abnormal root growth caused more than normal increase in dry weight when plants were treated for 2 days with 1 p.p.m.; however, this increase did not prove to be significant. At the end of the

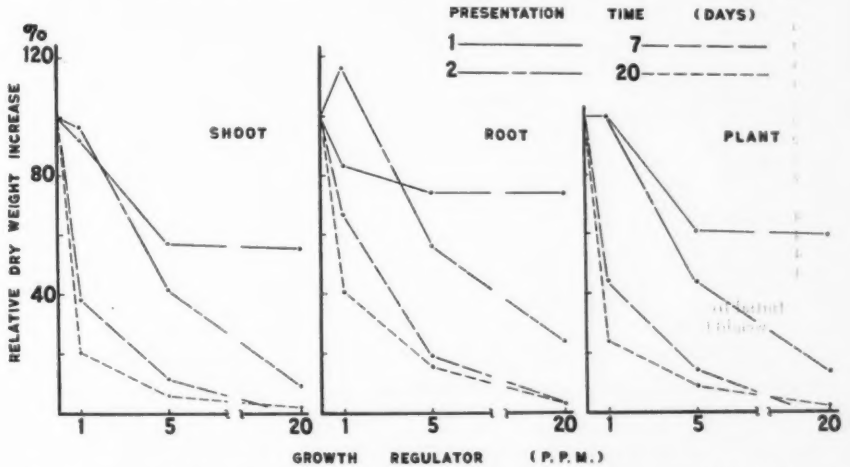

FIG. 2.—The relative increases in the dry weight of corn plants, which occurred during the 3d through the 5th weeks of growth when 2-week-old plants were exposed to 2,4-dichlorophenoxyacetic acid in nutrient solution for 1, 2, or 7 days or continuously. Increases in the dry weight of untreated plants, which are shown as 100 per cent, were 820, 270, and 1090 mg. for shoot, root, and total plant weight, respectively.

TABLE 3

THE FRESH AND DRY WEIGHTS OF 5-WEEK-OLD WHEAT PLANTS, TREATED AT THE MIDDLE OF THE THIRD WEEK OF GROWTH FOR 2, 4, 6, OR 17 DAYS WITH 1–4 P.P.M. OF 2,4-DICHLOROPHENOXYACETIC ACID. MEAN WEIGHTS ARE SHOWN FOR FOUR REPLICATES, EACH CONTAINING EIGHT PLANTS*

Concentration (p.p.m.)	Presentation time (days)	Fresh weight of:					
		Shoots		Roots		Total plant	
		Final (gm.)	Increase (%)	Final (gm.)	Increase %	Final (gm.)	Increase (%)
0	17	6.62	100	3.80	100	10.42	100
1	2	5.31	72	4.35	124	9.66	89
1	4	4.96	64	4.17	120	9.13	81
1	6	4.85	62	4.69	129	9.54	87
1	17	2.95	21	2.24	30	5.19	24
2	2	5.54	77	5.14	159	10.68	104
2	4	4.70	58	4.22	118	8.92	78
2	6	3.91	41	4.24	119	8.15	67
2	17	1.97	0	2.03	20	4.00	6
4	2	4.65	57	4.57	134	9.22	83
4	4	4.45	53	4.26	120	8.71	75
4	6	3.80	39	3.88	103	7.68	60
4	17	2.01	0	2.36	35	4.37	12
Initial fresh weight (gm.)		2.00		1.58		3.60	

Concentration (p.p.m.)	Presentation time (days)	Dry weight of:					
		Shoots		Roots		Total plant	
		Final (mg.)	Increase (%)	Final (mg.)	Increase (%)	Final (mg.)	Increase (%)
0	17	910	100	190	100	1100	100
1	2	750	77	202	110	952	82
1	4	645	62	205	112	850	70
1	6	660	64	242	142	902	76
1	17	260	7	120	43	380	13
2	2	715	72	232	134	947	82
2	4	580	53	200	108	780	61
2	6	470	37	200	108	670	48
2	17	200	0	92	20	292	2
4	2	600	56	205	112	805	65
4	4	595	55	225	128	820	66
4	6	480	39	212	116	692	63
4	17	210	0	90	19	300	3
Initial dry weight (mg.)		210		67		275	

* Minimum significant difference for means of shoot dry weight: At 19:1 odds: for the experiment, 125 mg.; for 2,4-dichlorophenoxyacetic acid treatments, 117 mg.

test the appearance of some plants grown continuously in 1 p.p.m. of 2,4-dichlorophenoxyacetic acid and of plants exposed more than 1 day to 5 and 20 p.p.m. strongly suggested that these plants would not continue to grow to maturity if transferred to normal nutrient solution. This test indicated that 2.5–7 days of exposure to concentrations of 2,4-dichlorophenoxyacetic acid of the order of 5–10 p.p.m. would cause more than 50 per cent reduction in growth of young corn plants.

Two-week-old wheat plants were treated continuously and for 2, 4, or 6 days with 1, 2, or 4 p.p.m. of 2,4-dichlorophenoxyacetic acid in a test which lasted 17 days. On the 12th day of the test period the roots of plants treated with 1 or 2 p.p.m. for more than 1 day were discolored, plainly showed inhibition of elongation and branching, and had localized swellings at the root tips. Symptoms developed by plants exposed to higher-intensity treatments differed mainly in that swelling was evident in the crown region. In general, the degree of these responses was proportional to the intensity of the treatment (presentation time × concentration).

At the end of the test period, shoots of plants which had grown in 4 p.p.m. for 6 days or continuously were partly dry. The green and dry weights of untreated plants increased approximately three and four times, respectively, compared to initial plant weights. The data on fresh and dry weights are summarized in table 3, and the relative average increases in dry weight are shown in figure 3. Except for the continuous treatments, which resulted in reductions of from 50 to 75 per cent, the increase in the weight of roots of treated plants was equal to that of control plants. However, roots were distinctly abnormal, swollen, and

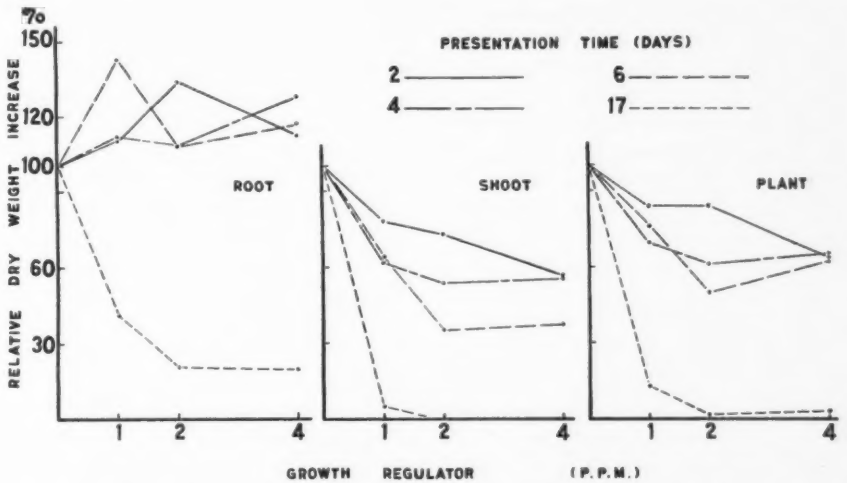

FIG. 3.—The relative increase in the dry weight of wheat plants during the period from the middle of the 3d through the 5th weeks of growth after 17-day-old plants were treated for 2, 4, 6, or 7 days with 1–4 p.p.m. of 2,4-dichlorophenoxyacetic acid in the nutrient solution. The average increases in the weight of root, shoot, and total plant—125, 700, and 825 mg., respectively—are valued as 100 per cent in each case.

soft, and much of the root weight increase was due to stimulated production of adventitious roots.

In proportion to the intensity of the treatments, all treatments caused parallel and significant reductions in dry weight of shoots, which constituted the major part of total plant weight. Continuous treatment with 1–4 p.p.m. resulted in at least 90 per cent reduction in dry weight of plants. When these concentrations were presented for 2 or 4 days, 25–50 per cent of the normal increase in dry weight of plants took place, and these plants appeared capable of further growth. These results suggest that wheat plants, especially with respect to root growth, are relatively tolerant to concentrations of 2,4-dichlorophenoxyacetic acid of the order of 5 p.p.m. presented for periods of time up to 6 days.

Discussion

The relatively greater effectiveness of 2,4-dichlorophenoxyacetic acid on broadleaf plants compared to monocotyledonous species was again borne out by results obtained in these presentation-time studies. Soybean plants were distinctly more inhibited by equivalent treatments with 2,4-dichlorophenoxyacetic acid than were corn or wheat plants. It appeared that in solutions which contained concentrations of the order of 5 p.p.m., soybeans would be killed or their growth completely arrested by exposure for slightly more than 2 days; whereas in similar concentrations appreciably more than 7 days' treatment was necessary to produce similar effects on corn and wheat.

Soybean plants treated for 7 days or longer with 5 p.p.m. died in less than 2 weeks after treatments were initiated, and no increase in dry weight occurred prior to their death. Though such treatments proved distinctly deleterious to the cereal crops, appreciable increases in the dry weight of plants did result. Treatment for 2 or 7 days with 5 or 1 p.p.m. of 2,4-dichlorophenoxyacetic acid, respectively, resulted in 40–60 per cent reduction of the increase in the dry weight of soybeans, 55 per cent reduction for corn, and, in comparable treatments, from 25 to 35 per cent reduction for wheat.

With respect to growth of roots, wheat plants proved relatively tolerant, and, indeed, increases in weight were induced by treatment with 2,4-dichlorophenoxyacetic acid, but these were due primarily to abnormal development. Such root growth was accompanied by abnormal swelling of the crown region. Both of these responses were again observed to occur to a much lesser extent in corn than in wheat plants (2). However, from the general appearance of plants subjected to comparable treatment, it appeared that wheat plants would be capable of a greater measure of recovery than corn.

In numerous tests conducted in the greenhouse and in the field as a part of other phases of the program on chemical regulation of the growth of plants, 2,4-dichlorophenoxyacetic acid was applied in various ways to the soil, and progressively diminishing amounts of activity were noted to persist in the soil for periods of from 3 to 6 weeks, depending upon the rate of treatment, the character of the soil, and the environmental complex (1). Although the development of plants in soil and in nutrient solutions is not identical, the observations reported in this paper as to effects of root exposure to 2,4-dichlorophenoxyacetic acid for limited periods may be applicable in considering possibilities of injury or recovery in soil in which persistence of the regulator is also limited.

Summary

Observations were made on the development of young vegetative soybean, corn, and wheat plants, which were subjected in nutrient solutions for 1, 2, 6, or 7 days to various concentrations of 2,4-dichlorophenoxyacetic acid. After the desired exposure period had elapsed, such plants were transferred to nutrient solution free of the regulator. Their growth during test periods was compared with that of untreated and continuously treated plants.

Inhibition of the growth of all three crops resulted from treatment for more than 2 days with 1 p.p.m. Abnormal types of growth of shoots and roots of plants occurred in all treatments. The responses induced in roots were more abnormal than were those in shoots.

The growth of cereals was distinctly less inhibited by equivalent treatments than that of soybeans. It appeared that treatment for slightly more than 2 days with 5 p.p.m. would kill or completely arrest the growth of soybeans. Seven days or more of treatment with 5 p.p.m. arrested the growth of the cereals. Wheat was slightly less inhibited than corn by equivalent treatments.

Significant differences in the amount of inhibition of growth resulted from variation of 2,4-dichlorophenoxyacetic acid concentration, length of presentation time, or treatment intensity (concentration × presentation time). Inhibition was generally proportional to treatment intensity. Exposure for 2 or 7 days to 5 or 1 p.p.m. reduced the increases in the dry weight of soybean plants by 40 and 60 per cent and that of corn by 55 per cent, while comparable treatments resulted in only 25–35 per cent reductions in the weight of wheat plants.

The increase in the weight of shoots of cereals remained nearly normal in low-intensity treatments and was reduced by higher-intensity treatments. More than normal increase in the weight of soybean shoots and plants occurred, following 1 day of treatment with 1 p.p.m.

In all plants 2,4-dichlorophenoxyacetic acid induced an abnormal type of root growth. This effect was at a maximum in low-intensity treatments, such as 1 or 2 days with 5 or 1 p.p.m., and, among the species tested, soybeans were the least affected. Low-intensity treatments significantly increased the dry weight of wheat roots and caused shoots to proliferate and swell at the crown regions. These responses were less marked in corn.

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OBSERVATIONS ON THE GROWTH OF CERTAIN PLANTS IN NUTRIENT SOLUTIONS CONTAINING SYNTHETIC GROWTH-REGULATING SUBSTANCES

III. THE RELATIVE TOXICITY OF ISOPROPYLPHENYLCARBAMATE AND SOME PHENOXYACETIC ACID DERIVATIVES TO SOME CEREALS[1]

D. L. TAYLOR, MAJ., A.U.S.

Introduction

A series of tests has been conducted with the object of comparing the effects of selected synthetic growth-regulators on the growth of cereals in nutrient-solution cultures. In earlier tests it had been determined that marked quantitative and morphological effects were induced when low concentrations of 2,4-dichlorophenoxyacetic acid were supplied in the nutrient solutions (**6**, **7**). Selected for comparison with 2,4-dichlorophenoxyacetic acid were: ammonium 2,4-dichlorophenoxyacetate and ammonium 2-methyl-4-chlorophenoxyacetate (both of which are more soluble in water than the respective acids) and isopropylphenylcarbamate (which is much less water-soluble). In other studies (**9**), the latter two chemicals showed activity differing from that of 2,4-dichlorophenoxyacetic acid. Isopropylphenylcarbamate, particularly, has high inhibitory activity on cereal seedlings (**1**).

Some effects of urethanes on the growth of plants have been reported by other investigators. FRIESEN (**4**) reported distinct effects of low concentrations of ethyl urethane (ethylcarbamate or $C_2H_5OOCNH_2$) on germination and early seedling development of oat and wheat plants. Ethylphenylurethane in low concentrations has been recently reported to have differential herbicidal effects (**8**). Cereals were killed or their growth distinctly arrested at all stages of development by a number of aryl carbamic esters and related compounds, which at the same rate of application were without marked effect on dicotyledonous plants. Isopropylphenylcarbamate, which was three times as active as ethylphenylcarbamate, had the greatest activity of the compounds tested.

In recent publications attention has been directed to the selective herbicidal action of 2-methyl-4-chlorophenoxyacetic acid, and its inorganic salts. This substituted acid was stated to be superior to 2,4-dichlorophenoxyacetic acid because of its more precisely selective toxicity to common broadleaf weeds(**3**, **5**).

Methods and materials

Experimental methods used in these tests were in all ways similar to those described in the first report of this series (**6**).

Experimental results

EXPERIMENT I

Comparison of the effects of 2,4-dichlorophenoxyacetic acid and isopropylphenylcarbamate on the growth of wheat.

Wheat plants were treated with 2,4-dichlorophenoxyacetic acid during the

[1] These studies were conducted at Camp Detrick, Frederick, Md., during March and April, 1945, under the supervision of Dr. A. G. Norman. The author gratefully acknowledges the able assistance given him by I. E. Freeman, Ph.M. 2/c, U.S.N.R., in conducting these investigations.

4th and 5th weeks of growth. Inhibition of growth of shoots was noticeable after 4 days of treatment with 1.5–3.0 p.p.m. of the acid. On the 6th day the shoots of the plants were chlorotic. Fresh-weight data, which are incomplete for these treatments, since most plants grown in 3 p.p.m. were dead and dry, indicate that, in proportion to the concentration supplied, less than 3 p.p.m. significantly limited the increase in fresh weight of shoots. At 0.05–1 p.p.m. the weight increases were from 80 to 10 per cent of the controls, while in greater concentrations the increases in the fresh weight of shoots were less than 5 per cent of normal.

The fresh weight of roots was significantly greater than normal in 0.10 p.p.m. or less; equal to untreated roots in concentrations up to 0.5 p.p.m.; and decreased to 70 per cent of normal when the concentration was increased to 2 p.p.m. The fresh weight of the total plant was significantly increased by the addition of 0.05 p.p.m. but was progressively decreased as the concentration of 2,4-dichlorophenoxyacetic acid was increased above this amount. Increment increases of 0.25–0.5 p.p.m. were required to result in significant progressive decreases (table 1). The minimum weight was at about 2 p.p.m.

A response of the root system which involved part or all of a series of five types of symptoms was first noticeable on the 3d day. It was distinct by the 6th day of treatment, by which time roots were dark colored in cultures containing more than 0.5 p.p.m. of 2,4-dichlorophenoxyacetic acid. A water-soaked, deteriorated appearance of roots increased in proportion to regulator concentration. The concentration ranges which resulted in development of different symptoms on the root systems of wheat plants were: (1) 0.05 p.p.m., and more: lessened elongation of white fibrous roots until, at 1 p.p.m., no root growth of this type occurred; (2) 0.1 p.p.m., and more: fewer externally visible, normal lateral roots until, at approximately 2 p.p.m., no new branches appeared; (3) 0.1–3 or 4 p.p.m.: development of localized swellings on all root tips, which response was at a maximum at approximately 1.5 p.p.m. and was progressively less in higher concentrations until little swelling occurred in 4 p.p.m. or more; (4) 0.75–4 p.p.m.: stimulated adventitious root growth from the crown regions of plants, which reaction reached a maximum at 2–3 p.p.m. and again was lacking at 4–5 p.p.m.; (5) 1.5–10 p.p.m.: swelling of the crown region, most evident on plants treated with 5 p.p.m. of 2,4-dichlorophenoxyacetic acid. Appreciable crown swelling was caused by 10 p.p.m. before plants were killed.

All isopropylphenylcarbamate concentrations tested limited the increase in fresh weight of wheat treated from the middle of the 3d through the middle of the 5th weeks of growth. The responses of plants and plant parts were similar in the various concentrations, in that fresh weight decreased sharply as concentration was increased from 0.075 to 0.25 p.p.m. Further increase in concentration did not result in further decrease in the fresh weight of plants.

The root systems of plants treated with isopropylphenylcarbamate concentrations greater than 0.075 p.p.m. were discolored. Inhibition of normal elongation and branching was not directly correlated with concentration above 0.25 p.p.m., which was the distinct threshold. In proportion to concentrations of from 0.25 to 5 p.p.m., distinctly less swelling of root tips resulted in comparison with equal concentrations of 2,4-dichlorophenoxyacetic acid. Treatment with iso-

TABLE 1

THE FRESH AND DRY WEIGHTS OF WHEAT PLANTS WHICH WERE GROWN DURING THE 3D THROUGH THE 5TH WEEKS IN NUTRIENT SOLUTIONS CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID OR ISOPROPYLPHENYLCARBAMATE. WEIGHTS ARE MEANS FOR EIGHT PLANTS IN EACH OF QUADRUPLICATE CULTURES TREATED WITH 2,4-DICHLOROPHENOXYACETIC ACID (A) AND OF TRIPLICATE CULTURES TREATED WITH ISOPROPYLPHENYLCARBAMATE (B). CURVES 1 AND 2 IN FIGURE 1 SHOW THE DRY-WEIGHT DATA PRESENTED IN THIS TABLE UNDER A AND B, RESPECTIVELY.

REGULATOR (P.P.M.)	FINAL FRESH WEIGHT (GM.)*						PER CENT INCREASE IN FRESH WEIGHT					
	Shoots		Roots		Total		Shoots		Roots		Total	
	A	B	A	B	A	B	A	B	A	B	A	B
0.0	6.31	24.77	2.69	14.8c	9.00	39.57	100	100	100	100	100	100
0.05	5.55	.....	3.74	.....	9.29	.....	81	.....	184	.....	106	.....
0.075	.....	21.36	.....	13.74	.....	35.10	.....	85	.....	91	.....	87
0.10	4.11	.....	3.28	.....	7.38	.....	45	.....	145	.....	69	.....
0.25	3.31	6.69	2.72	4.07	6.03	10.76	25	17	102	6	44	13
0.50	3.41	7.36	2.74	4.41	6.15	11.77	28	20	104	9	46	16
0.75	2.87	5.83	2.52	3.96	5.39	9.79	14	12	87	5	32	10
1.00	2.70	5.64	2.61	3.90	5.31	9.53	10	12	94	4	30	10
1.50	2.25	6.86	2.38	4.29	4.63	11.15	0	18	75	8	17	14
2.00	2.42	.....	2.33	.....	4.74	.....	3	.....	72	.....	19	.....
2.50	.....	6.42	.....	4.69	.....	11.11	.....	16	.....	11	.....	14
5.00	Dead	5.33	Dead	4.28	Dead	9.61	Dead	11	Dead	7	Dead	10
Initial fresh weight (gm.)	2.32	3.01	1.41	3.44	3.73	6.45	.....	.....	.....	.....	.....	.....

REGULATOR (P.P.M.)	FINAL DRY WEIGHT (MG.)*						PER CENT INCREASE IN DRY WEIGHT					
	Shoots		Roots		Total		Shoots		Roots		Total	
	A	B	A	B	A	B	A	B	A	B	A	B
0.00	690	3187	113	753	803	3940	100	100	100	100	100	100
0.05	640	.....	138	.....	778	.....	89	.....	142	.....	95	.....
0.075	.....	3490	.....	763	.....	4253	.....	111	.....	102	.....	110
0.10	520	.....	128	.....	648	.....	63	.....	125	.....	70	.....
0.25	393	1497	143	379	535	1890	35	43	150	38	49	39
0.50	415	1580	143	427	558	2007	40	42	150	43	53	42
0.75	358	1290	136	353	493	1643	27	31	138	30	40	31
1.00	353	1170	150	360	503	1530	26	27	164	32	42	28
1.50	288	1447	125	390	413	1843	12	37	121	37	25	37
2.00	308	.....	125	.....	433	.....	17	.....	123	.....	29	.....
2.50	.....	1397	.....	390	.....	1787	.....	35	.....	37	.....	36
3.00	248	.....	113	.....	360	.....	4	.....	100	.....	15	.....
4.00	255	.....	105	.....	360	.....	5	.....	88	.....	15	.....
5.00	238	983	105	340	343	1323	1	20	88	28	11	22
10.00	245	.....	75	.....	320	.....	3	.....	38	.....	7	.....
Initial dry weight (mg.)	233	430	53	180	286	610	.....	.....	.....	.....	.....	.....

* Minimum significant difference in mean weight at 19:1 odds: Fresh weight (gm.): shoots (A) 0.88; roots (A) 0.58; total plant (A) 0.13, (B) 4.15. Dry weight (mg.): shoots (A) 131; roots (A) 40; total plant (A) 121, (B) 610.

propylphenylcarbamate resulted in no stimulation of adventitious root growth and relatively little swelling of the crown region of plants.

The response of wheat plants to isopropylphenylcarbamate differed sharply from that caused by 2,4-dichlorophenoxyacetic acid, in that all plants treated with the former remained alive throughout the test period. Growth of shoot was distinctly inhibited by more than 0.075 p.p.m. However, the degree of inhibition was not distinctly proportional to concentration above 0.25 p.p.m. Although this substance inhibited growth of the main shoot, it also appeared to stimulate the production of small tillers. The maximum number of these occurred at less than 0.25 p.p.m. Shoots of plants were distinctly dark green, appeared highly cutinized, and were stiff or leathery to the touch.

Complete dry-weight data (table 1) showed that, except for growth of roots in 2,4-dichlorophenoxyacetic acid solution, in no case was there a significant increase in total weight or weight of plant parts. More than 0.10 p.p.m. of either compound significantly decreased the dry weight of all plant parts. Isopropylphenylcarbamate limited growth slightly more than 2,4-dichlorophenoxyacetic acid in the concentration range 0.10–1.0 p.p.m. (fig. 1); but when the concentration range was increased above 2 p.p.m., the added increments of the latter depressed growth relatively more than similar added increments of the former. Very low concentrations (0.075 p.p.m.) of isopropylphenylcarbamate stimulated tiller growth. Abnormal growth of wheat-roots was stimulated by 2,4-dichlorophenoxyacetic acid concentrations up to 2 p.p.m.

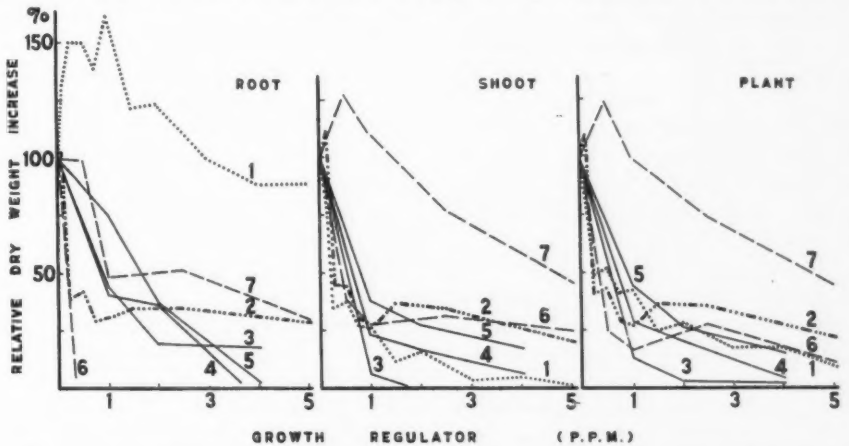

FIG. 1.—The relative increases in the dry weights of wheat and rice plants grown continuously in nutrient solutions containing growth-regulators: Curves are labeled: (for expt. I on wheat) *1*, 2,4-dichlorophenoxyacetic acid; *2*, isopropylphenylcarbamate; (for expt. II on wheat) *3*, 2,4-dichlorophenoxyacetic acid; *4*, ammonium 2-methyl-4-chlorophenoxyacetate; *5*, ammonium 2,4-dichlorophenoxyacetate; (for expt. III on rice) *6*, 2,4-dichlorophenoxyacetic acid; and *7*, isopropylphenylcarbamate. The dry-weight increases in the absence of growth-regulator are valued as 100 per cent.

EXPERIMENT II

Comparison of the effects of 2,4-dichlorophenoxyacetic acid, ammonium 2,4-dichlorophenoxyacetate, and ammo-

nium 2-methyl-4-chlorophenoxyacetate on the growth of wheat.

Several lots of wheat plants, 2 weeks old, growing in nutrient solution, were treated with concentrations of 1, 2, or 4 p.p.m. of 2,4-dichlorophenoxyacetic acid, ammonium 2,4-dichlorophenoxyacetate, or ammonium 2-methyl-4-chlorophenoxyacetate. All plants receiving 1 or 4 p.p.m. and half of the plants receiving 2 p.p.m. of the respective growth-regulators were exposed continuously for 17 days to these concentrations. The other half of the plants supplied with solutions containing 2 p.p.m. were grown for only 4 days in these solutions and were then removed to normal nutrient solution and grown there for 13 days more.

At the end of the 17-day test period all plants were alive. Parts of shoots were dry in all cultures containing 4 p.p.m. of any one of the growth-regulators. In those treated continuously with 2 p.p.m. of 2,4-dichlorophenoxyacetic acid or ammonium 2-methyl-4-chlorophenoxyacetate, parts of shoots were similarly dry. Roots in all cultures on continuous treatment containing 2 p.p.m. or more of any of the growth-regulators were in poor condition. It appeared likely that a few of the plants treated with 4 p.p.m., and some treated continuously with 2 p.p.m., would be able to grow to maturity.

In proportion to concentration, all three growth-regulators caused darkening of the root systems and swelling of root tips and the crown regions of plants and stimulated the production of adventitious roots. 2,4-Dichlorophenoxyacetic acid, ammonium 2-methyl-4-chlorophenoxyacetate, and ammonium 2,4-dichlorophenoxyacetate ranked in decreasing order in these respects.

Among the cultures treated with 1 p.p.m., only 2,4-dichlorophenoxyacetic acid caused crown swelling. Roots of plants grown in ammonium 2,4-dichlorophenoxyacetate were relatively branched and elongated. The roots of plants grown in ammonium 2-methyl-4-chlorophenoxyacetate were the least discolored of those in 2-p.p.m. treatments, while ammonium 2,4-dichlorophenoxyacetate caused the greatest stimulation of adventitious root growth. Little stimulation of adventitious roots was evident in any culture treated with 4 p.p.m. The much-inhibited root systems of plants treated with 2-methyl-4-chlorophenoxyacetate showed slightly less deterioration than those of plants exposed to 4 p.p.m. of the other regulators.

Shoots of plants exposed for only 4 days to 2 p.p.m. of the growth-regulators were green at the end of the test period. In all cases the root systems were slightly discolored, and slight swelling of the root tips was evident. Only the 4-day treatment with 2,4-dichlorophenoxyacetic acid resulted in marked swelling of crowns. Crowns of plants treated with 2-methyl-4-chlorophenoxyacetate were slightly swollen. Similar treatments with ammonium 2,4-dichlorophenoxyacetate and ammonium 2-methyl-4-chlorophenoxyacetate stimulated the branching and elongation of roots compared to untreated and 2,4-dichlorophenoxyacetic acid-treated plants. The most stimulation resulted from ammonium 2,4-dichlorophenoxyacetate. At the end of the test it appeared that plants treated for 4 days with 2 p.p.m. of these growth-regulators would be able to continue growth to maturity.

Since parts of some treated plants were dry and since changes in the green weights of plants subjected to these growth-regulators were quite similar to the increases in dry weights, only dry-weight data are presented for this test

(table 2). The weight of shoots and plants was significantly decreased by all regulators in proportion to the concentration used, except in the 4-day treatments with ammonium 2,4-dichlorophenoxyacetate and ammonium 2-methyl-4-chlorophenoxyacetate. Root growth was stimulated with all three of the growth-regulators in the 4-day treatments. The greatest stimulation was caused by ammonium 2,4-dichlorophenoxyacetate. Among the 4-day treatments the least stimulation and most abnormal type of root growth was caused by 2,4-dichlorophenoxyacetic acid. All the continuous treatments decreased the weight of roots in proportion to concentration of regulator.

Increase in the dry weight of shoots was inhibited slightly more than increase in weight of roots by these chemical regulators (fig. 1). In general, 2,4-dichlorophenoxyacetic acid concentrations caused greater inhibition of growth than did equal treatments with either of the ammonium salts. The amount of growth which occurred in the ammonium 2,4-dichlorophenoxyacetate treatments was notably greater than that in equivalent concentrations of 2,4-dichlorophenoxyacetic acid or ammonium 2-methyl-4-chlorophenoxyacetate.

TABLE 2

THE DRY WEIGHT OF WHEAT PLANTS GROWN DURING THE 3D THROUGH THE 5TH WEEKS IN NUTRIENT SOLUTIONS CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID (3), AMMONIUM 2-METHYL-4-CHLOROPHENOXYACETATE (4), OR AMMONIUM 2,4-DICHLOROPHENOXYACETATE (5). MEAN WEIGHTS ARE FOR FOUR REPLICATES, EACH CONTAINING EIGHT PLANTS

Regulator tested	Concentration (p.p.m.)	Presentation time (days)	Shoots		Roots		Total	
			Final weight (mg.)*	Increase (%)	Final weight (mg.)*	Increase (%)	Final weight (mg.)*	Increase (%)
Control	0	.....	910	100	190	100	1100	100
5	2	4	800	84	295	185	1095	99
4	2	4	690	69	255	153	945	81
3	2	4	580	53	200	108	780	61
5	1	17	465	36	160	75	625	42
	2	17	370	23	110	35	480	25
	4	17	330	17	70	3	400	15
4	1	17	385	25	115	39	500	27
	2	17	325	16	110	35	435	19
	4	17	250	6	60	0	310	5
3	1	17	260	7	120	43	380	13
	2	17	200	0	95	20	295	2
	4	17	210	0	90	19	300	3
Initial weight (mg.)	.....	.....	210	.....	67	.....	275	.....

* The minimum significant difference in mean shoot weight for treated plants is 85 mg. The minimum significant difference in mean total plant weight for 4-day treatments with 2 p.p.m. (only) is 255 mg.

Experiment III

Comparison of the effects of 2,4-dichlorophenoxyacetic acid and isopropyl-

phenylcarbamate singly or in mixtures on the growth of rice plants.

Rice plants which had been grown in sand for 9 weeks were mounted in solution cultures containing 0.00022 M. calcium nitrate, 0.00012 M. monopotassium acid phosphate, 0.00012 M. magnesium sulphate, 0.00004 M. ammonium sulphate; and the following p.p.m. concentrations of minor elements: 0.5 of iron, 0.25 of boron, 0.25 of manganese, 0.01 of cobalt, and 0.025 of zinc. When the plants were 10 weeks old, the cultures were supplied with this nutrient solution with the addition of 0.5, 1.0, 2.5, or 5.0 p.p.m. of 2,4-dichlorophenoxyacetic acid or isopropylphenylcarbamate. Cultures were also treated with 1-, 2.5-, and

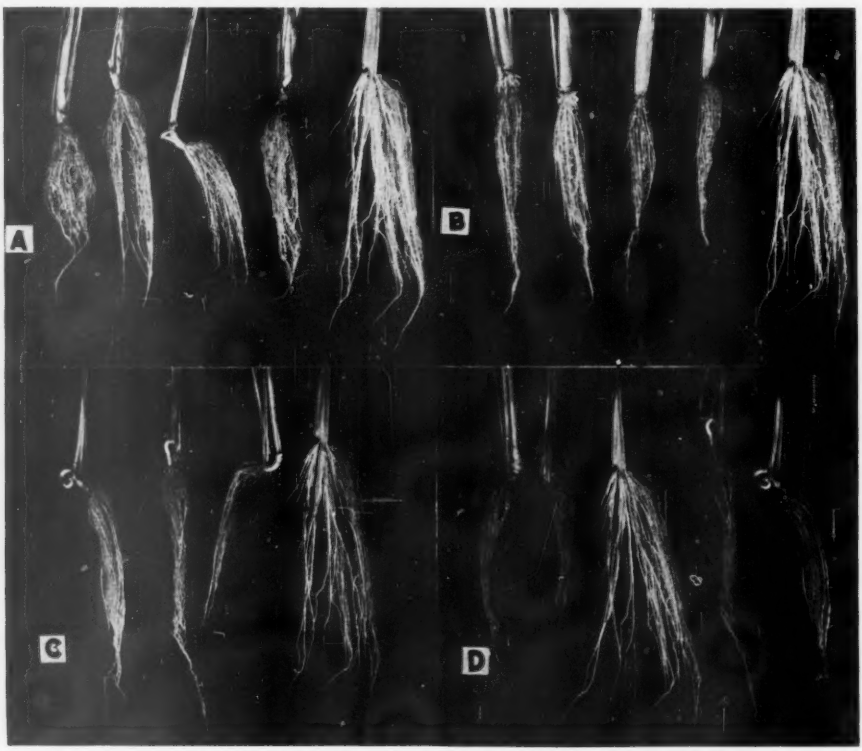

FIG. 2.—Root systems and the crown regions of rice plants, 14 weeks old, grown continuously after the 10th week in nutrient solutions containing growth-regulators. From left to right the treatments supplied to plants shown were *A*, 0.5, 1.0, 2.5, 5.0 p.p.m. of 2,4-dichlorophenoxyacetic acid, and control. (Note: on treated plants, dark color, inhibition of elongation and branching of roots, swollen crowns; and swollen root tips in 0.5 p.p.m.) *B*, 0.5, 1.0, 2.5, 5.0 p.p.m. of isopropylphenylcarbamate, and control. (Note: stimulated production of adventitious roots in 0.5–2.5 p.p.m., slightly swollen crowns, and the slightly darker color of treated roots.) *C*, 1.0-, 2.5-, and 5.0-p.p.m. mixtures of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid in 1:3 proportion, and control. (Note: dark color, and decreased elongation and branching of roots; few adventitious roots, and swollen crowns on treated plants.) *D*, 1.0- and 2.5-p.p.m. mixtures of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid in 1:1 proportion, and control, and 2.5- and 1.0-p.p.m. mixtures of these agents, respectively, in 1:3 proportion. (Note: less swelling of the crown region and more growth of adventitious roots on the treated plant at the right.)

5-p.p.m. mixtures of these growth-regulators in the proportions 1:3, 1:1, and 3:1.

These older rice plants developed symptoms which, in general, were similar to those described for wheat (expts. I and II), in response to equivalent concentrations of 2,4-dichlorophenoxyacetic acid in the nutrient solution. All plants were alive at the end of the 4-week test period. Parts of shoots were dry, and the swelling of crowns was much decreased in cultures which contained 2.5 p.p.m. or more of this regulator.

All treatments caused discoloration of roots and inhibited their elongation. Maximum typical swelling of root tips and crowns was caused by treatments with 0.5–1.0 p.p.m. and by 1.0–2.5 p.p.m. of 2,4-dichlorophenoxyacetate acid, respectively. No swelling of root tips and little or no swelling of the crowns of plants occurred in solutions containing isopropylphenylcarbamate. Plants treated with 0.5 and 1.0 p.p.m. of this regulator developed abnormal numbers of adventitious roots.

The general appearance of plants treated with mixtures of these regulators indicated that the effect of 2,4-dichlorophenoxyacetic acid predominated over that of isopropylphenylcarbamate. Stimulation of tillering and the development of stunted, dark-green, leathery leaves, similar to the responses noted when wheat was treated with isopropylphenylcarbamate, were not evident in rice plants. Some of the changes which were induced by these treatments are evident on the representative 14-week-old plants which appear in figures 2 and 3.

The data on dry weight (table 3) indicate that 2,4-dichlorophenoxyacetic acid treatment resulted in distinctly greater inhibition of the vegetative growth of rice than equivalent concentrations of isopropylphenylcarbamate. All treatments, except 0.5 and 1.0 p.p.m. of isopropylphenylcarbamate, caused significant inhibition of the growth of rice plants or plant parts (fig. 1). Shoot growth was significantly increased by 0.5 p.p.m. of isopropylphenylcarbamate, which did not alter the weight of roots from normal. One p.p.m. of isopropylphenylcarbamate decreased the weight of roots but did not affect the increase in weight of shoots.

FIG. 3.—Crown regions and portions of the root systems of rice plants treated with 1 p.p.m. of 2,4-dichlorophenoxyacetic (left) or isopropylphenylcarbamate (right). (Note: differences in color and general vigor of the root systems, swollen deteriorated crown of the plant on the left, and greater growth of adventitious roots from the crown region of the plant on the right.)

Treatment with any of the various mixtures of growth-regulators did not result in more pronounced effects on the growth of plants than those caused by equivalent concentrations of 2,4-dichlorophenoxyacetic acid alone. These data indicate possible, but only slight, supplemental inhibitory effects of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid supplied in mixtures. The amount of inhibition of rice growth

in any mixture most nearly corresponded to that which resulted in 2,4-dichlorophenoxyacetic acid equivalent to the concentration of that component in the mixture.

Discussion

In concentrations as low as 0.25–1.0 p.p.m., all substances tested were highly active in affecting the growth of cereals in nutrient-solution culture. Low concentrations, which resulted in general growth inhibition, also stimulated marked changes in the character of the root systems. Swelling of root tips of cereals in these tests closely resembled the morphological response of the roots of sweet peas, the shoots of which were treated with 4-chlorophenoxyacetic acid (**2**).

The ability of cereals to accomplish a relatively great amount of such abnormal root growth may, in part, account for the

TABLE 3

THE AVERAGE DRY WEIGHTS OF RICE PLANTS GROWN IN NUTRIENTS CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID (6), ISOPROPYLPHENYLCARBAMATE (7), OR MIXTURES OF THESE REGULATORS. MEAN WEIGHTS ARE OF QUADRUPLICATE CULTURES, EACH CONTAINING FOUR PLANTS

Growth-regulator treatment				Shoots		Roots		Total	
(7) (p.p.m.)	(6) (p.p.m.)	Total (p.p.m.)	Proportion (7):(6)	Final weight (mg.)	Increase (%)	Final weight (mg.)	Increase (%)	Final weight (mg.)	Increase (%)
Control	.....	0.0	.....	5040	100	1578	100	6620	100
.....	0.5	0.5	.....	2832	38	753	0	3585	25
.....	1.0	1.0	.....	2388	26	778	0	3165	16
.....	2.5	2.5	.....	2645	32	993	3	3638	28
.....	5.0	5.0	.....	2385	25	1043	12	3428	23
0.5	.....	0.5	.....	6023	127	1575	99	7598	124
1.0	.....	1.0	.....	5360	109	1258	48	6618	99
2.5	.....	2.5	.....	4260	78	1283	52	5543	74
5.0	.....	5.0	.....	3115	45	1138	28	4253	43
0.25	0.75	1.0	1:3	2385	24	938	0	3323	20
0.50	0.50	1.0	1:1	2488	27	848	0	3335	20
0.75	0.25	1.0	3:1	2555	29	1023	9	3578	26
0.63	1.87	2.5	1:3	2225	20	828	0	3053	13
1.25	1.25	2.5	1:1	2008	14	845	0	2853	9
1.87	0.63	2.5	3:1	2245	21	973	1	3218	18
1.25	3.75	5.0	1:3	2048	15	968	0	3015	13
2.50	2.50	5.0	1:1	2405	25	983	2	3388	22
Initial weight (mg.)	.....	.....	.....	1532	.....	969	.....	2501	.....

	Shoots	Roots	Total
Minimum significant difference of mean weight (mg.) at odds of:			
99:1	930	505	1290
19:1	690	380	965

differential action of soil treatments with substituted phenoxyacetic acids. Maintained during such treatment periods by abnormal growth of the root systems, stimulated production of adventitious roots, and abnormal crown development, cereals could later, to greater extent, resume and continue more normal growth.

These tests did not clearly indicate any marked difference in the toxicity of isopropylphenylcarbamate and 2,4-dichlorophenoxyacetic acid to growth of young cereal plants. Increase in the amount of growth of shoots occurred in very low concentrations of the former, while with from 0.25 to 2.0 p.p.m. the growth of wheat was inhibited slightly more than with 2,4-dichlorophenoxyacetic acid. Two p.p.m. or more limited the growth of wheat slightly less than equivalent concentrations of 2,4-dichlorophenoxyacetic acid. The latter inhibited the growth of rice more than isopropylphenylcarbamate in all concentrations tested.

The appearance of plants which were treated with these growth-regulators was distinctly different, in that 2,4-dichlorophenoxyacetic acid treatments resulted in parts of shoots withering or dying; whereas, throughout the test periods, the shoots arrested by isopropylphenylcarbamate remained alive, becoming dark-green and highly cutinized or leathery. Such response to isopropylphenylcarbamate has been previously reported by others (**8**), who also describe a crown-swelling response, which occurred following treatment of cereals with this substance. Little swelling of crowns resulted from treatments with this regulator in nutrient solution, but low concentrations stimulated the production of small tillers on wheat and of adventitious roots on older rice plants.

Recently it has been suggested that 2-methyl-4-chlorophenoxyacetic acid was more precisely selective when used as a herbicide to control growth of weeds growing with cereals (**3**). In these nutrient-solution tests the ammonium salt of this growth-regulator appeared slightly, and in some instances significantly, less toxic to wheat than equivalent treatments with 2,4-dichlorophenoxyacetic acid. Of the three phenoxyacetic acid derivatives studied, ammonium 2,4-dichlorophenoxyacetate was the least toxic to cereals.

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OBSERVATIONS ON THE GROWTH OF CERTAIN PLANTS IN NUTRIENT SOLUTIONS CONTAINING SYNTHETIC GROWTH-REGULATING SUBSTANCES

IV. THE AMOUNT OF GROWTH IN SOIL AND SOLUTION CULTURES TREATED WITH EQUAL WEIGHTS OF AMMONIUM 2,4-DICHLOROPHENOXYACETATE[1]

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Introduction

In the course of the work described in the preceding papers (**3**, **4**, **5**), it was observed that phenoxyacetic acids presented in nutrient-solution cultures caused greater inhibition to the growth of plants than was caused by equal or larger amounts of such growth-regulators applied as soil treatments. Studies were therefore made to determine the magnitude of the influence of soil on the action of ammonium 2,4-dichlorophenoxyacetate by supplying equal weights of this growth-regulator in soil and in solution cultures.

Methods and materials

The soil cultures consisted of 1-gallon glazed crocks, each of which contained an air dry weight of 3770 gm. of thoroughly mixed sand, compost, and field topsoil in the proportions of 1:1:2. The soil moisture level in these cultures was maintained at 32.7 per cent (on the basis of the air dry weight of the soil mixture, or 1235 gm./culture) by weighing the cultures twice daily and adding water as required. An equal volume was maintained in nutrient-solution cultures by adding twice daily the required amounts of distilled water.

The solution-culture crocks were filled with fresh nutrient solution just prior to the time when the growth-regulator was added. Thereafter no more nutrient was added to the cultures, which were aerated twice daily during the test period. The nutrient solution and, in all other respects, the management of these cultures were the same as those described in the first paper of this series (**3**).

When the plants were 2 weeks old and established in the soil and solution cultures, aliquots of growth-regulator stock solution containing the desired weight of regulator were evenly applied to the surface of the soil or thoroughly mixed with the solution cultures. The regulator was placed in the soil cultures just prior to the addition of water, in order that the water might carry the regulator solution down through the soil. Quadruplicate cultures of each type, which contained three red kidney beans or soybeans or eight oat plants, were treated with 2.5, 5, and 10 mg. of ammonium 2,4-dichlorophenoxyacetate. These amounts were calculated to give theoretical initial concentrations of approximately 2, 4, or 8 p.p.m. The plants were allowed to grow for a period of 8 days after treatment, at which time data on dry weight were obtained.

[1] This study was conducted at Camp Detrick, Frederick, Md., during April, 1945, under the supervision of Dr. A. G. Norman. The author gratefully acknowledges the able assistance given him by I. E. Freeman, Ph.M. 2/c, in conducting these investigations.

Results

During the test period, responses of root systems similar to those previously described for treatments with ammonium 2,4-dichlorophenoxyacetate (**5**) were observed on all three species in nutrient solutions containing the regulator. Swelling of the hypocotyl region and stimulated growth of adventitious roots occurred on all kidney beans treated in solution cultures and on plants in soil cultures which were treated with 10 mg. The root systems of all plants were much stunted in all solution-culture treatments. All treatments of soybeans and oats resulted in only slight swelling of the hypocotyl and crown region.

Applications of 8 p.p.m. to solution cultures resulted in death of kidney beans and soybeans by the 6th day, while oat plants similarly treated were in poor condition. All plants were dead or partly dry in solution cultures containing 4 p.p.m., and even at 2 p.p.m. in solution culture some of the kidney beans and soybeans were dead. The plants in soil cultures presented a sharp contrast in appearance. Only when 10 mg. of ammonium 2,4-dichlorophenoxyacetate was added was there swelling or drying of parts of the plants. Most of the kidney beans and soybeans in the other treatments and all oat plants survived and at the end of the experiment appeared to be capable of continued growth.

The dry-weight data (table 1) indicate that the growth of these crops in soil or solution cultures was inhibited by all treatments in proportion to the concentration of the growth-regulator applied. In comparison to the effects in solution cultures, the effects of the several treatments in soil cultures were distinctly less acute. In general, the relative growth of plants in the light soil used was approximately four to five times that which oc-

TABLE 1

THE EFFECT OF EQUAL AMOUNTS OF AMMONIUM 2,4-DICHLOROPHENOXYACETATE APPLIED IN SOIL AND IN SOLUTION CULTURES ON THE GROWTH OF OATS, KIDNEY BEANS, AND SOYBEANS. VALUES ARE MEANS OF SHOOT DRY WEIGHT OF PLANTS (EIGHT OAT, THREE KIDNEY BEAN, OR THREE SOYBEAN PLANTS) IN QUADRUPLICATE CULTURES*

Regulator treatment		Soil		Solution	
Mg.	P.p.m.	Final	Increase†	Final	Increase†
		Gm.	%	Gm.	%
		Oat culture.			
0.0	0.0	2.30	100	1.59	100
2.5	2.0	2.22	96	0.73	19
5.0	4.0	1.98	83	0.69	15
10.0	8.0	1.62	64	0.75	21
Initial weight (gm.)	.....	0.42		0.53	
		Kidney bean cultures			
0.0	0.0	3.07	100	0.98	100
2.5	2.0	2.09	64	0.43	5
5.0	4.0	1.18	30	0.34	0
10.0	8.0	0.57	8	0.28	0
Initial weight (gm.)*	.....	0.36		0.40	
		Soybean cultures			
0.0	0.0	1.34	100	0.30	100
2.5	2.0	0.88	61	0.17	35
5.0	4.0	0.83	57	0.13	15
10.0	8.0	0.53	31	0.11	6
Initial weight (gm.)*	.....	0.17		0.10	

* Values shown for kidney beans and soybeans represent the average weight of the trifoliate leaves only.

† For evaluation of the relative increases in weight, that of untreated plants in each type of culture is valued at 100 per cent.

curred in solution cultures containing equal amounts, or theoretical concentrations, of ammonium 2,4-dichlorophenoxyacetate. Conversely, the relative amount of growth of plants in treated cultures was approximately the same when the amount of growth-regulator applied to soil cultures was four times that applied in solution cultures. In these tests, run concurrently, oats were the least affected and kidney beans the most inhibited by the treatments applied either to soil or in nutrient cultures.

The growth of wheat, clover, and sugar-beet plants in that order was recently reported to be increasingly affected by soil treatment with 2,4-dichlorophenoxyacetic acid. In a light, sandy soil which was low in organic matter, this growth-regulator markedly inhibited the development of these crops. The growth of clover, and especially that of wheat, was much less arrested when equivalent treatments were given in soil high in organic matter (2). Similar effects of organic matter on the toxicity of this regulator to kidney beans and other crops were observed in the general program of these investigations (1). It seems probable that some such relationship obtained in the light-soil mixture employed in the comparative tests described herein and that, in a soil of higher organic and clay content, the differences in the intensity of the responses produced in soil and nutrient culture might be even greater. No explanation is yet available for the apparent retention or inactivation of the growth-regulator by the organic or colloid components of the soil. The observations reported above raise the possibility of controlling water plants by applications that might not affect other plants growing in soil along the margins.

Summary

Red kidney beans, soybeans, and oat plants, 2 weeks old, were grown in soil and solution cultures which contained equal volumes of water and were treated with equal weights of ammonium 2,4-dichlorophenoxyacetate.

Morphological changes in plants were induced and growth of all species was arrested by all treatments in proportion to the concentration of the regulator supplied.

The effects of equivalent treatments on these crops increased in the following order: oats, soybeans, and kidney beans. Oats were distinctly less affected than the broadleaved species.

When equivalent amounts of ammonium 2,4-dichlorophenoxyacetate were supplied, approximately four to five times as much inhibition of growth occurred in solution cultures as in cultures containing the light-soil mixture used in these tests.

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GENERAL INDEX

A

Alkaloid content of Ecuadoran cinchona barks 184

Allard, R. W., Action of isopropylphenylcarbamate upon plants 589; Effects of certain growth-regulating compounds on Irish potatoes 568; Some effects of plant growth-regulators on seed germination and seedling development 575

Allium, cytological effects of sulfanilamide 390

Altitude, and radial growth of trees 462; effect on composition of Derris 467

Ammonium 2,4-dichlorophenoxyacetate, effect on plant growth in soil and solution cultures 630

Andropogon furcatus, chemical composition 427

Apple scald, affected by growth-regulating substances 284

B

Barley, seed germination in soil containing 2,4-dichlorophenoxyacetic acid 408

Beal, J. M., Histological reactions of bean plants to certain of the substituted phenoxy compounds 200; book reviews 137, 296

Bean, affected by 2,4-dichlorophenoxyacetic acid 332, 532, 552; histological responses, 200, 312, 522; movement of 2,4-dichlorophenoxyacetic acid stimulus 393, 509

Beets, translocation of reproductive stimulus 86

Beloperone, cystoliths and plasmodesmata 372

"Bibliography of references to the literature of the minor elements" 296

Bisby, G. R., "Introduction to the taxonomy and nomenclature of fungi" 296

Black, C. A., Effect of commercial fertilizers on sex expression of hemp 114

Boehmeria, cystoliths and plasmodesmata 372

Bonner, James, Further investigation of toxic substances which arise from guayule plants 343

Boron requirement, relation of photoperiod 454

Borthwick, H. A., Interaction of nitrogen nutrition and photoperiod as expressed in bulbing and flower-stalk development of onion 52; Relationship of photoperiod and nitrogen nutrition to initiation of flower primordia in soybean 218

Boyd, F. T., Effect of plant growth-regulators in relation to stages of development of certain dicotyledonous plants 563; Effects of certain growth-regulating compounds on Irish potatoes 568; Influence of rainfall on the effectiveness of 2,4-dichlorophenoxyacetic acid sprayed for herbicidal purposes 540; Response of kidney bean and soybean plants to aqueous-spray applications of 2,4-dichlorophenoxyacetic acid with and without carbowax 552

Britton, M. E., "A catalog of Illinois algae" 137

Brown, J. W., Effect of 2,4-dichlorophenoxyacetic acid on the readily available carbohydrate constituents in annual morning-glory 120; Effect of 2,4-dichlorophenoxyacetic acid on the water relations, the accumulation and distribution of solid matter, and the respiration of bean plants 332; Movement of 2,4-dichlorophenoxyacetic acid stimulus and its relation to food materials in plants 393

Bryophyllum, cicatrization in leaves 95

C

Carbohydrate constituents in annual morning-glory affected by 2,4-dichlorophenoxyacetic acid 120

Carbowax in spray applications of 2,4-dichlorophenoxyacetic acid 552

Carlson, Margery C., Megasporogenesis and development of the embryo sac in Cypripedium parviflorum 107

Carrots, enzyme content 362

Cereals, effect of isopropylphenylcarbamate 589; relative toxicity of some growth-regulating substances 620

Chemical composition, of Derris, effect of water supply and altitude 467; of grasses, effect of season, habitat, and clipping 527

Cicatrization in leaves of Bryophyllum calycinum 95

Cinchona barks, alkaloid content 184

Conifer seedlings, effects of plant growth-regulators 139, 268, 297

Cuttings of leaves, propagation of Taraxacum koksaghyz 260

Cypripedium, embryo sac 107, 291

Cystoliths, in Beloperone, Ficus, and Boehmeria 372

Cystopsora oleae, development of spore-forms 74

Cytological effects of sulfanilamide on Allium 390

Cyto-taxonomic studies in Oryzopsis 1

D

Daubenmire, R. F., Radial growth of trees at different altitudes 462

Davis, F. F., Herbicial properties of 2,4-dichlorophenoxyacetic acid applied in dusts containing hygroscopic agents 129

DeRose, H. R., Absorption and translocation of 2,4-dichlorophenoxyacetic acid 509; Action of isopropylphenylcarbamate upon plants 589; Persistence of some plant growth-regulators when applied to the soil in herbicidal treatments 583; Some effects of plant growth-regulators on seed germination and seedling development 575

Derris elliptica, composition 467

Dichlorophenoxyacetic acid (2,4), effect in nutrient solutions 597; effect in relation to plant development 532, 563; effect of exposure time 611; effect

on carbohydrate constituents 120; effect on growth of grass 276, 417; effect on seed germination 352, 408; effect on water relations, solid matter, and respiration 332; effect on woody plants 379; herbicidal properties 62, 129, 379, 540, 544; histological responses in bean 522; method for determination of low concentrations 507; movement of stimulus 393; 509; spray applications with and without carbowax 552

E

Eaton, S. V., book review 296

Eggers, Virginia, Influence of carbohydrate and nitrate-nitrogen nutrition on development of hypocotyledonary buds in flax 385; Mode, site, and time of initiation of hypocotyledonary bud primordia in Linum usitatissimum 441

Embryo sac, Cypripedium 107, 291

Ennis, W. B., Jr., Action of isopropylphenylcarbamate upon plants 589; Effect of plant growth-regulators in relation to stages of development of certain dicotyledonous plants 563; Effects of certain growth-regulating compounds on Irish potatoes 568; Response of kidney bean and soybean to aqueous-spray applications of 2,4-dichlorophenoxyacetic acid with and without carbowax 552

Eny, D. M., Organic acids of lemon fruits 231

Enzymes, of carrots 362

F

Ficus, cystoliths and plasmodesmata 372

Flax, hypocotyledonary buds 385, 441

Food materials in plants, relation of 2,4-dichlorophenoxyacetic acid 120, 393

Fritsch, F. E., "Structure and reproduction of the algae. Vol. II" 137

G

Gandara, J. A., Alkaloid content of Ecuadoran and other American cinchona barks 184

Gorham, P. R., Investigations on rubber-bearing plants 260

Grasses, effect of season, habitat, and clipping on chemical composition 427; growth as affected by 2,4-dichlorophenoxyacetic acid 276, 417

Growth-regulating substances, effect in relation to plant development 532, 563, 568; effect on apple scald 284; effect on growth of grasses 276, 417, 589; effect on growth of seedlings 139, 268, 297, 352, 575; effect on plant constituents 120, 332; effect on water relations, solid matter, and respiration 332; herbicidal properties 129, 379, 540, 544, 552, 560, 589; histological responses 62, 200, 312, 522; in nutrient solutions 597, 611, 620, 630; methods and tests 129, 476, 507, 552, 560; movement of stimulus 393, 509; new compounds 475, 476; seed germination 352, 408, 575; soil treatment 352, 408, 583, 630

Guayule, toxic substances 343

H

Hamner, C. L., Effect of treating soil and seeds with 2,4-dichlorophenoxyacetic acid 352; Herbicidal action of 2,4-dichlorophenoxyacetic acid on several shrubs, vines, and trees 379; Histological reactions in bindweed and sow thistle following herbicidal treatments 62

Hemp, effect of commercial fertilizers on sex expression 114

Herbicidal properties, of isopropylphenylcarbamate 583, 589, 620; of 2,4-dichlorophenoxyacetic acid 62, 129, 352, 379, 408, 540, 544, 560, 583

Histological reactions to plant growth-regulators, in bean plants 200, 312, 522; in bindweed and sow thistle 62

Höber, Rudolph, "Physical chemistry of cells and tissues" 295

Horsfall, J. G., "Fungicides and their action" 296

Hypocotyledonary buds in flax 385, 441

I

Imhofe, Barbara, Histological reactions in bindweed and sow thistle following herbicidal treatments 62

Indolebutyric acid, effects on survival of tree seedlings 268

Isopropylphenylcarbamate, action upon plants 589; persistence in soil 583; relative toxicity 620

J

Johnson, B. L., Cyto-taxonomic studies in Oryzopsis 1

K

Kraus, E. J., Margaret MacLeod vii

L

Landes, Margaret L., Investigations on rubber-bearing plants 260

Lemon fruits, organic acids 231

Lineweaver, Hans, Seasonal variation in the enzyme content of carrots 362

Link, G. K. K., Mode, site, and time of initiation of hypocotyledonary bud primordia in Linum usitatissimum 441; book review 296

Linum usitatissimum, hypocotyledonary buds 385, 441

M

MacLeod, Margaret, obituary sketch vii

MacVicar, Robert, The relation of photoperiod to the boron requirement of plants 454

Maize ear, methods for studying 425

Maki, T. E., Effects of naphthaleneacetic-acid sprays on the development and drought resistance of pine seedlings 297; Effects of soaking with indolebutyric acid on root development and survival of tree seedlings 268

Marshall, H., Effects of naphthaleneacetic-acid sprays on the development and drought resistance of pine seedlings 297; Effects of soaking with indolebutyric acid on root development and survival of tree seedlings 268

Marth, P. C., Effect of growth-regulating substances on development of apple scald 284; Effect of spray

mixtures containing 2,4-dichlorophenoxyacetic acid, urea, and fermate on the growth of grass 417; Effects of 2,4-dichlorophenoxyacetic acid on growth of grass plants 276; Germination of seeds in soil containing 2,4-dichlorophenoxyacetic acid 408; Herbicidal properties of 2,4-dichlorophenoxyacetic acid applied in dusts containing hygroscopic agents 129

Martin, W. E., Alkaloid content of Ecuadoran and other American cinchona barks 184

Minarik, C. E., Influence of rainfall on the effectiveness of 2,4-dichlorophenoxyacetic acid sprayed for herbicidal purposes 540

Mitchell, J. W., Effect of 2,4-dichlorophenoxyacetic acid on the readily available carbohydrate constituents in annual morning-glory 120; Effects of spray mixtures containing 2,4-dichlorophenoxyacetic acid, urea, and fermate on the growth of grass 417; Effects of 2,4-dichlorophenoxyacetic acid on growth of grass plants 276; Germination of seeds in soil containing 2,4-dichlorophenoxyacetic acid 408; Herbicidal properties of 2,4-dichlorophenoxyacetic acid applied in dusts containing hygroscopic agents 129; Movement of 2,4-dichlorophenoxyacetic acid stimulus and its relation to food materials in plants 393

Moore, R. H., Some effects of altitude and water supply on the composition of Derris elliptica 467

Morris, H. J., Seasonal variation in the enzyme content of carrots 362

Moulton, J. E., Effect of treating soil and seeds with 2,4-dichlorophenoxyacetic acid 352

Murray, Mary A., Carpellary and placental structure in the Solanaceae 243; Histological responses of bean plants to phenylacetic acid 312

Mustard, seed germination in soil containing 2,4-dichlorophenoxyacetic acid 408

N

Naphthaleneacetic acid, effects on pine seedlings 297

Nemesia strumosa, flower pigmentation 32

Nitrogen nutrition, effect on flower initiation in soybean 218; interaction with photoperiod in onion 52

Norman, A. G., New growth-regulating compounds. I. Summary of growth-inhibitory activities of some organic compounds 476; studies in plant growth-regulating substances 475

Nutrient solutions, containing growth-regulating substances 597, 611, 620, 630

Nutrition, influence on development of hypocotyledonary buds in flax 385

O

Onion, cytological effects of sulfanilamide 390; interaction of nitrogen nutrition and photoperiod 52

Oryzopsis, cyto-taxonomic studies 1

Ostrom, C. E., Effects of naphthaleneacetic-acid sprays on the development and drought resistance of pine seedlings 297; Effects of plant growth-regulators on shoot development and field survival of forest-tree seedlings 139

P

Parker, M. W., Interaction of nitrogen nutrition and photoperiod as expressed in bulbing and flower-stalk development of onion 52; Relationship of photoperiod and nitrogen nutrition to initiation of flower primordia in soybean 218

Peters, J. J., Cytological effects of sulfanilamide on Allium cepa 390

Phenoxy compounds, effect on bean plants 200; relative toxicity of some derivatives 620

Phenylacetic acid, histological response in bean 312

Photoperiod, effect on flower initiation in soybean 218; interaction with nitrogen nutrition in onion 52; relation to boron requirement 454

Pigmentation, anthocyanin and flower pattern in Nemesia 32

Pine seedlings, effects of naphthaleneacetic acid 297

Plasmodesmata in Beloperone, Ficus, and Boehmeria 372

Potatoes, effects of growth-regulating compounds 568

R

Rayner, M. C., "Trees and toadstools" 137

Reeves, R. G., Methods for studying the maize ear 425

Reproductive stimulus, translocation in sugar beets 86

Riley, H. P., Inheritance of the main anthocyanin pigmentation and of some of its patterns in flowers of Nemesia strumosa 32

Root development of tree seedlings affected by indolebutyric acid 268

Rubber-bearing plants 260

S

Schomer, H. A., Effect of growth-regulating substances on development of apple scald 284

Scott, Flora M., Cystoliths and plasmodesmata in Beloperone, Ficus, and Boehmeria 372

Scully, N. J., Interaction of nitrogen nutrition and photoperiod as expressed in bulbing and flower-stalk development of onion 52; Relationship of photoperiod and nitrogen nutrition to initiation of flower primordia in soybean 218

Seed germination, effects of plant growth-regulators 352, 408, 575

Sex expression in hemp affected by commercial fertilizers 114

Sinclair, W. B., Organic acids of lemon fruits 231

Smith, H. H., Quantitative aspects of aqueous spray applications of 2,4-dichlorophenoxyacetic acid for herbicidal purposes 544

Soil treated with growth-regulating substances 352, 408, 583, 630

Solanaceae, carpellary and placental structure 243

Soybean, as affected by 2,4-dichlorophenoxyacetic acid 532, 552; initiation of flower primordia affected by photoperiod 218

Steward, F. C., book reviews 137, 295

Stipa spartea, chemical composition 427

Stout, M., Translocation of the reproductive stimulus in sugar beets 86

Struckmeyer, B. Esther, The relation of photoperiod to the boron requirement of plants 454

Sulfanilamide, cytological effects on Allium 390

Swamy, B. G. L., Embryo sac and fertilization in Cypripedium 291

Swanson, C. P., Effect of plant growth-regulators in relation to stages of development of certain dicotyledonous plants 563; Effects of certain growth-regulating compounds on Irish potatoes 568; Histological responses of kidney bean to aqueous sprays of 2,4-dichlorophenoxyacetic acid 522; New growth-regulating compounds. I. Summary of growth-inhibitory activities of some organic compounds 476; A simple bio-assay method for the determination of low concentrations of 2,4-dichlorophenoxyacetic acid in aqueous solutions 507; Some effects of plant growth-regulators on seed germination and seedling development 575; Two methods for the determination of the herbicidal effectiveness of plant growth-regulating substances in oil solution 560

T

Taraxacum kok-saghyz, propagation by leaf cuttings 260

Taylor, D. L., Observations on the growth of certain plants in nutrient solutions containing growth-regulating substances. I. Some effects of 2,4-dichlorophenoxyacetic acid 597; II. The influence of presentation time 611; III. The relative toxicity of isopropylphenylcarbamate and some phenoxyacetic acid derivatives to some cereals 620; IV. The amount of growth in soil and solution cultures treated with equal weights of ammonium 2,4-dichlorophenoxyacetate 630

Thirumalachar, M. J., Development of spore-forms and the nuclear cycle in Cystopsora oleae 74

Thompson, H. E., New growth-regulating compounds. I. Summary of growth-inhibitory activities of some organic compounds 476

Toxic substances from guayule 343

Trees, radial growth at different altitudes 462; seedlings affected by plant growth-regulators 139, 268, 297

Tukey, H. B., Effect of treating soil and seeds with 2,4-dichlorophenoxyacetic acid 352; Herbicidal action of 2,4-dichlorophenoxyacetic acid on several shrubs, vines, and trees 379; Histological reactions in bindweed and sow thistle following herbicidal treatments 62

W

Water supply, effect on composition of Derris 467

Weast, C. A., Seasonal variation in the enzyme content of carrots 362

Weaver, R. J., Absorption and translocation of 2,4-dichlorophenoxyacetic acid 509; Action of isopropylphenylcarbamate upon plants 589; Effect of plant growth-regulators in relation to stages of development of certain dicotyledonous plants 563; Effect of spray applications of 2,4-dichlorophenoxyacetic acid on subsequent growth of kidney-bean and soybean plants 532; Influence of rainfall on the effectiveness of 2,4-dichlorophenoxyacetic acid sprayed for herbicidal purposes 540; Some effects of season, habitat, and clipping on the chemical composition of Andropogon furcatus and Stipa spartea 427

Welch, W. B., Cicatrization in leaves of Bryophyllum calycinum 95

Whiting, A. Geraldine, Histological responses of bean plants to phenylacetic acid 312

Woody plants, effects of 2,4-dichlorophenoxyacetic acid 379